PROPERTIES OF AQUEOUS-ALCOHOL-WASHED PROTEIN CONCENTRATES PREPARED FROM AIR-CLASSIFIED PEA PROTEIN AND OTHER AIR-CLASSIFIED PULSE PROTEIN FRACTIONS

A Thesis Submitted to the

College of Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Food and Bioproduct Sciences

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

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2018

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ABSTRACT

Pea protein concentrates were prepared from air-classified pea protein by aqueous-alcohol (ethanol or isopropanol) washing. Response surface methodology (Box Behnken design) was used to create mathematical models to explain quantitatively the relationship between treatment combinations (aqueous alcohol concentration, extraction temperature, extraction time) on the protein contents and yields of the pea protein concentrates. Also studied were the effects of these treatments on the starch, fat, lipid, ash and oligosaccharide contents and the functionality (water hydration capacity (WHC), oil hydration capacity (OHC), emulsion activity (EA), emulsion stability (ES), foaming capacity (FC), foam stability (FS) and nitrogen solubility index (NSI) of the concentrates.

The protein contents of concentrates decreased as the concentration of alcohol increased, whereas yield increased. Time and temperature were found to have no significant effect on protein content or yield. The protein contents and yields of aqueous-ethanol and isopropanol washed concentrates ranged from 68.2-72.1%, 66.4-76.1% 66.6-73.1% and 63.9-76.4%, respectively. Optimal conditions for protein content were identified as 52% aqueous-ethanol, 32°C, 12-minute extraction time or 55% aqueous-isopropanol, 50°C, 11-minute extraction time. Optimal conditions for yield were identified as 65% aqueous-ethanol, 40°C, 11-minute extraction time or 70% aqueous-isopropanol, 44°C, 10-minute extraction time. All aqueous-alcohol-washed concentrates were true protein concentrates (protein concentration >65% on a dry weight basis) and were higher in protein and starch and lower in lipid and raffinose-family oligosaccharides in comparison to the starting material, air-classified pea protein.

In general, the aqueous-alcohol-washed concentrates exhibited higher functionality values compared to the starting material, and WHC, OHC, EA, ES, FC and FS were similar for corresponding concentrates prepared using aqueous-ethanol or aqueous-isopropanol. In a few instances, aqueous-ethanol and aqueous-isopropanol had differential effects on ES, FC and/or FS. NSI was affected negatively by all treatments. All aqueous-alcohol-washed concentrates were lighter in colour and more green (less red) and more blue (less yellow) than the starting material. The functionality of aqueous-alcohol-washed concentrates was similar in most respects to that of one or both of the commercial soy concentrates analyzed.

The optimal conditions identified for protein and yield of aqueous-alcohol-washed pea concentrates were used in the preparation of concentrates from air-classified pea, fababean, lentil and navy bean protein fractions. Only products prepared from air-classified pea and fababean protein contained over 65% protein (dry weight basis) and could be classified as true protein concentrates, due to the lower protein contents of the lentil and navy bean air-classified protein fractions. The effects of aqueous-ethanol washing on the composition and functionality of products prepared from fababean, lentil and navy bean were similar to those observed previously for aqueous-alcohol-washed pea protein concentrates.

The aqueous-ethanol extracts obtained from preparation of protein concentrates from air-classified pea protein at conditions identified as optimal for protein content or yield were analyzed for their composition. The extracts were similar in composition and contained lipid, protein and oligosaccharide components. Stachyose was the most abundant oligosaccharide, and raffinose the least abundant. The protein constituents of the extracts were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. The starting material, concentrates and extracts contained proteins in the molecular mass range of 10-95 kDa, 17-95 kDa and 10-20 kDa, respectively. The concentrates were depleted in lower molecular mass components; these components were predominant in the extracts.

Fat was extracted from pea and chickpea flours using either hexane or 70%-aqueous-ethanol prior to fine grinding and air classification to determine the effects of fat removal on the yield and composition of air-classified fractions. Hexane was the more effective solvent for fat removal. For both pea and chickpea, extraction of fat prior to air classification reduced the yield of the fine (protein-enriched) fraction and increased the protein content of both the coarse (starch-rich) and fine fractions. For chickpea, air classification was much more effective in separating starch and protein when fat-reduced flours were employed.

A product prepared by Agriculture and Agri-Food Canada from air-classified pea protein by reflux extraction with 80%-aqueous-ethanol was analyzed for its composition and functionality. The reflux-extracted product was similar in composition to aqueous-ethanol-washed pea concentrates and commercial concentrates from soybean, with the exception of its lower protein and higher oligosaccharide contents. The reflux-extracted product was similar in functionality to aqueous-ethanol-washed concentrates, with the exception of its lower NSI.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my most sincere gratitude to my major supervisor, Dr. Robert Tyler, for his constant guidance and encouragement. He served beyond his capacities as a supervisor and mostly as a father figure throughout my program. I would like to thank my advisory committee, Drs. Venkatesh Meda (co-supervisor), Michael Nickerson and Thomas Warkentin, as well as past and present committee chairs, Drs. George Khachatourians and Xiao Qiu, for their invaluable insight and feedback.

I especially would like to thank Dr. Michael Nickerson for the multiple employment opportunities he provided that helped me gain better insight into the functionalities of plant proteins.

I sincerely appreciate the efforts of Dr. Lope Tabil who helped me understand and rationalize my statistical data.

I greatly appreciate the assistance received during sample analysis from Erin Labbie and Tracey Yeo at Parrheim Foods, Peter Frohlich for his help in working with the air-classifier at Cigi in Winnipeg, Manitoba and Dr. Shannon Hood Neifer for help with statistics.

In addition, I would like to acknowledge Dr. Manu Pratap Gangola from the Department of Plant Sciences for his helping me with RFO analyses and Dr. Ravi Chibbar for permitting me to use his laboratory.

I would like to thank the faculty, staff and students in the Department of Food and Bioproduct Sciences for their support, friendship and insightful discussions. Special thanks to Kornsulee Ratanapariyanuch who served as a friend and a technical backbone, providing assistance and training for many analyses. Thank you to Heather Silcox, Yuanlong Cao and Jianheng Shen for their technical assistance, and to the administrative support staff, Ann Harley and Donna Selby, for handling all of my miscellaneous inquiries.

Last, but definitely not least, I would like to thank the Saskatchewan Ministry of Agriculture, Agriculture and Agri-Food Canada, and Parrheim Foods for providing financial support or materials for my research.

DEDICATION

This thesis is firstly dedicated to God, for it is by his grace that I made it so far. To mom, dad and my amazing husband who were always a source of encouragement and support.
And finally, Dr. R.T. Tyler, who has been a constant source of kindness, knowledge and inspiration.

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LIST OF SYMBOLS AND ABBREVIATIONS

a*	Red-green colour, with red associated with higher values of a*
AAFC	Agriculture and Agri-Food Canada
AACCI	American Association of Cereal Chemists International
ACPP	Air-classified pea protein
b*	Yellow-blue colour, with yellow associated with higher values of b*
°C	Degrees Celsius
DFSF	Defatted soy flour
d.b.	Dry basis
EA, ES	Emulsion activity, emulsion stability
FAO	Food and Agriculture Organization
FC, FS	Foaming capacity, foam stability
g	Gram
g	Gravity
GMO	Genetically Modified Organism
h	Hour
IEP	Isoelectric precipitation
kDa	KiloDalton
L*	Brightness, 0 to 100, moving from dark to light
LFSF	Low-fat soy flour
min	Minute
mL	Millilitre
m^3/h	Cubic metres per hour

mg	Milligram
nm	Nanometre
NSI	Nitrogen Solubility Index
OHC	Oil holding capacity
pI	Isoelectric point
PPI	Pea protein isolate
PF	Pea flour
PPF	Pea protein fraction
PPI	Pea protein isolate
PTF	Pea flour made from hulls
PDI	Protein dispersibility index
PNaI	Pea protein isolate as sodium proteinate
RFO	Raffinose family oligosaccharide
RO	Residual oil
rpm	Revolutions per minute
sec	Second
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
UF	Ultrafiltration
v/v	Volume to volume
w/v	Weight to volume
w/w	Weight to weight
WHC	Water hydration capacity
µm	Micrometre
µL	Microlitre

1. INTRODUCTION

Pulses are plants belonging to the family *Leguminosae* which are cultivated worldwide for their seed, and they constitute an important source of food and feed protein (Duranti & Gius, 1997). The family *Leguminosae* is second in economic importance to the *Poaceae* (grass/cereal) family (Anonymous, 2006). The Food and Agriculture Organization of the United Nations defines pulses as leguminous plants primarily harvested for their seed (FAO, 1994), e.g. dry bean, chickpea, lentil and pea. Additionally, pulses can be classified into 11 groups as follows: dry beans (which include kidney, pinto, navy, adzuki, mung, black gram, scarlet runner, rice bean, moth and tepary beans), dry broad beans (which include horse, broad and field bean), dry peas, chickpeas, black-eyed peas, pigeon peas, lentils, bambara groundnut, vetch, lupins, and other 'minor' pulses (jack, winged, velvet and yam beans (Nikmaram et al., 2017).

Pulses are nutrient-rich foods and provide energy, dietary fibre, protein, minerals and vitamins required for human health (Anonymous, 2014). Nutrition makes a significant contribution to the cause, prevention and progression of disease (Rebello, Greenway & Finley, 2014). Published data provides testimony that consumption of pulses may have potential health benefits, including reduced risk of HIV, cardiovascular diseases, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal diseases and reduction of LDL cholesterol. Such studies have contributed to a growing awareness of the desirability of including pulses in the diet (Mudryj, Yu & Aukema, 2014; Boye, Zare & Pletch, 2010; Nikmaram et al., 2017).

Alternative ingredient sources high in protein and fibre and/or having gluten-free status are being sought as replacements for animal protein. Pulses are gradually gaining importance as they play a significant role in alleviation of protein-energy malnutrition (Butt & Batool, 2010; Kudre, Benjakul and Kishimura, 2013). Soybean protein has been the major product for many years now, but concerns related to allergenicity, high phytic acid content, GMO status, and its content of goitrogenic compounds and phytoestrogens have resulted in growing demand for other solutions. As a result, pulses have grown in popularity as protein-rich food ingredients that can change health

outcomes and improve nutrition (Anonymous 2014). Commercial processes employed for the preparation of protein concentrates from soy, such as alkaline extraction, isoelectric washing and aqueous-alcohol washing, are not well suited to pulse flours due to their high starch content.

Air classification is a process for the separation of mixed dry materials on the basis of their physical properties, including particle size, shape, density and aerodynamic characteristics (Chrismon, 1978). It is a dry process that has been employed for the manufacture of starch-rich and protein-rich fractions from pulses. Particle size is the primary basis for separation; the process concentrates protein in a fine fraction and starch in a coarse fraction. Separation is enhanced by the round, smooth, dense nature of starch granules and the light more jagged nature of protein-rich particles. The protein-rich fraction resulting from air classification of pulse flours generally contains only 50-60% protein, thus such products do not qualify as true protein concentrates, which by definition must contain at least 65% protein on a moisture-free basis (Youngs, 1975; Tyler, 1982; Sosulski, 1983; Wright, Bumstead, Coxon, Ellis, DuPont, & Chan, 1984; Horváth, Ormai-Cserhalmi & Czukor, 1989; Pokatong, 1994). The protein-rich fractions also retain strong flavour and suffer from oxidative instability. However, air-classified protein-rich fractions, due to their low starch content, may serve as good starting materials for preparation of protein concentrates by aqueous-alcohol washing.

Pokatong (1994) demonstrated that protein concentrates in high yield, essentially devoid of unwanted colour, flavour and lipid, and substantially lower in trypsin inhibitor activity could be produced by aqueous-alcohol washing of air-classified pea protein. With the exception of 90% and 95% aqueous-ethanol-washed products, all concentrates qualified as true protein concentrates.

The primary objectives of this research were: i) to optimize the aqueous-alcohol washing process for preparation of protein concentrates from air-classified pea protein fractions using aqueous-ethanol and aqueous-isopropanol; ii) to use the aqueous-ethanol washing parameters identified as optimum for pea for the preparation of protein concentrates from air-classified fractions of pea, lentil, fababean and navy bean, and compare the products with pea-derived products; iii) to determine the composition, potential utilization and value of the aqueous-ethanol extract; iv) to examine the effect of washing pea and chickpea flours with aqueous-ethanol or hexane prior to air classification on the yield and composition of the air-classified starch and

protein fractions and v) to evaluate the composition and functionality of a product prepared by reflux extraction of air-classified pea protein with 80%-aqueous-ethanol.

1.2 Hypotheses

The following hypotheses were tested:

- Optimal conditions with respect to concentrate yield and protein content for the preparation of protein concentrates containing 65-70% or more of protein from air-classified pea protein by aqueous-alcohol washing can be identified.
- Protein concentrates containing 65-70% or more of protein can be prepared by aqueous-ethanol washing of air-classified protein fractions from lentil, fababean and navy bean.
- The extract obtained by aqueous-ethanol washing of air-classified pea protein fractions will exhibit interesting and potentially useful composition and functionality.
- Aqueous-ethanol or hexane washing of pea and chickpea flours prior to air classification will increase the efficiency of the starch-protein separation.
- The product resulting from reflux extraction of air-classified pea protein with 80%-aqueous-ethanol would have potential for use as a protein concentrate in food or feed applications.

1.3 Objectives

The following were the objectives of this research project:

- To determine the optimum conditions (alcohol concentration, extraction temperature and extraction time) with respect to concentrate yield and protein content for preparation of protein concentrates by aqueous-ethanol and aqueous-isopropanol washing of air-classified pea protein.

- To compare the yield, composition and functionality of protein concentrates prepared by aqueous-ethanol washing of air-classified protein fractions from pea, lentil, fababean and navy bean.
- To determine the composition and potential utility and value of the extract obtained from aqueous-ethanol washing of air-classified pea protein.

- To study the effect of aqueous-ethanol and hexane washing of pea and chickpea flours prior to air classification on protein separation efficiency and the composition of the air-classified fractions.
- To determine the functionality of a product prepared by reflux extraction of air-classified pea protein with 80%-aqueous-ethanol.

2. LITERATURE REVIEW

2.1 Constituents of pulses

The FAO defines pulses as legumes harvested particularly for their dry grain (FAO, 2016). Pulses are dietetic powerhouses due to their rich nutritional profile. They are high in fibre, which accounts for the reduced glycemic response despite their high carbohydrate content, good sources of protein and energy, low in fat, and are packed with micronutrients (Rebello, Greenway & Finley, 2014). Pulses serve as a vital dietary protein source for a large segment of the world's population (Boye, Zare & Pletch, 2010). Pulses can be described as containing approximately 10% moisture, 18-32% protein, 1-6% lipid, 60-65% total carbohydrate and 2.5-4% ash (Dalgetty, Baik & Swangson, 2003; Tiwari & Singh, 2012; Rebello, Greenway & Finley, 2014; Boye, Zare & Pletch, 2010; Coxon & Wright, 1985; Rachwa-Rosiak, Nebesny, & Budryn, 2015; Takayama, Muneta, & Wiese, 1965). Pulses also contain substantial amounts of micronutrients such as Vitamins A, B and E and minerals such as selenium, iron and zinc (Winham & Hutchins, 2007). The chemical composition of field pea, lentil, fababean, bean and chickpea will be described in more detail in this section.

2.1.1 Chemical composition

2.1.1.1 Protein

Pulses are good sources of protein. Protein from pulses serves as a source of constructive compounds, energy and nutritionally essential amino acids, play bioactive roles, and act as precursors of biologically active peptides. The cotyledons in pulse seeds serve as the major protein source and make the highest contribution to overall protein content (Tiwari & Singh, 2012; Duranti, 2006). Pulses accumulate large amounts of protein during their growth and development, which is stored in membrane-bound organelles, the embryonic axis, and storage vacuoles or protein bodies in the parenchyma cells of the cotyledons, with a small amount in the seed coat (Tiwari & Singh, 2012; Duranti, 2006). The proteins in pulses are of two types enzymatic-(metabolic) and structural. The majority of the protein in pulse seeds is in the form of

storage protein which, based on its solubility characteristics, is classified as albumin, globulin, prolamin or glutelin (Osborne & Campbell, 1898). Globulins, soluble in dilute salt solution, represent approximately 70% of the total protein in pulses. Globulins are classified on the basis of their sedimentation coefficients (S) as vicilin, which sediments at 7S, or legumin, which sediments at 11S, and these fractions usually predominate (Boye, Zare & Pletch, 2010).

Albumins account for 10-20% of the total protein and are soluble in water. Glutelins soluble in dilute acid or alkali account for 10-20% of the total protein in pulse seeds (Tiwari & Singh, 2012; Roy, Boye & Simpson, 2010). The non-storage proteins include enzymes, enzyme inhibitors, hormones, and transporting, structural and recognition proteins (Mosse & Pernollet, 1983).

Pea genotypes are reported to have protein contents ranging from 23-30% (Nx6.25). Albumins and globulins represent 15-25% and 50-60% of the total protein, respectively (Boye, Zare & Pletch, 2010; Guegen & Barbot, 1988). Pea seed storage proteins are composed mainly of legumin (11S), vicillin (7S) and albumins (2S), and pea protein isolates contain mainly globular (11S and 7S) proteins (Gatehouse et al., 1982; O'Kane et al., 2005). The ratio of legumin to vicilin in pea ranges from 0.2 to 1.5 (Casey et al., 1982). Pea protein products have been reported to exhibit functionality comparable and complementary to that of analogous soybean protein products. The protein content of chickpea ranges from 20-25% with albumin, globulin, prolamin and glutelin contributing 8-12%, 53-60%, 3-6% and 19-24%, respectively (Boye, Zare & Pletch, 2010). The storage globulins of fababean fall into two classes, legumin and vicillin. Together these proteins contribute approximately 20% of the mature seed dry weight (Ersland et al., 1983). In bean varieties, proteins are predominantly salt soluble globulins and include vicillin as the major fraction, with a minor fraction of legumin (Rui et al., 2011). Lentil exhibits protein contents in the range of 25-28%. The protein fractions in lentil include albumin, legumin, vicillin, glutenin and prolamin; these fractions have been found to be glycosylated (Boye, Zare, & Pletch, 2010).

2.1.1.1.1 Amino acid composition

The essential amino acid composition of pulse protein exhibits considerable variation. The lysine contents of pulse proteins tend to be high compared to that of soybean protein, and the contents of sulphur-containing amino acids (methionine and cystine) and tryptophan tend to be lower (Norton et al., 1985; Jansman, 1996). Differences in amino acid composition also can be

observed among the protein fractions of the seeds. Globulins are relatively deficient in sulphur-containing amino acids. Albumins are enriched in sulphur-amino acids and other essential amino acids (such as lysine) in comparison to globulins. Additionally, it has been found that some antinutritional factors, mainly proteinase inhibitors, contain a substantial amount of cystine and methionine. For example, common beans have been reported to have 30-40 % of the total seed cystine present in protease inhibitors (Baudoin, & Maquet 1999; Norton et al., 1985).

Pulses are deficient in the sulphur-containing amino acids and tryptophan, but are rich in lysine (Salunkhe, Kadam & Chavan, 1985). Amino acid composition data for field pea seed was summarized by Orr & Watt (1968), the Food and Agriculture Organization (1970) and Harvey (1970). Holt & Sosulski (1979) stated that arginine, leucine, lysine, aspartic acid and glutamic acid occurred in the highest amounts, and accounted for 50% of the total amino acids, whereas histidine, methionine, threonine, tryptophan and cystine accounted for less than 11%. Chickpea and fababean have been found to contain relatively high amounts of arginine, leucine, lysine, aspartic acid and glutamic acid, but fababean is lower in methionine than chickpea or field pea (Boye, Zare & Pletch, 2010; Kaldy & Kasting, 1974). The essential amino acid compositions of pea, lentil, fababean, bean and chickpea are shown in Table 2.1.

Table 2. 1. Essential amino acid compositions of various pulses (g/16 g N).

Amino Acid	Field Pea	Lentil	Fababean	Bean	Chickpea
Arginine	9.5	14	10.5	6.9	10.3
Histidine	2.3	3.9	2.6	3.2	3.4
Isoleucine	7.4	9.5	4.3	5.3	4.1
Leucine	6.9	15.8	8.3	9.0	7.0
Lysine	7.2	12.6	6.6	7.7	7.7
Methionine	1.0	1.6	0.7	1.3	1.6
Phenylalanine	4.6	10.6	4.2	6.0	5.9
Threonine	3.8	7.5	3.3	4.9	3.6
Tryptophan	0.8	ND	1.0	1.6	1.1
Valine	4.6	11.6	3.9	5.9	3.6

(Boye, Zare & Pletch, 2010; Salunkhe, Kadam & Chavan, 1985; Adams et al.,1985)

2.1.1.2 Carbohydrate

Pulses have a lower level of carbohydrate than cereals (Tiwari & Singh, 2012). Carbohydrate, according to its function in plants, can be classified into three groups- the mono- and disaccharides are sources of energy for growth, the oligosaccharides and starch are storage carbohydrates, and pectins, hemicelluloses and other non-cellulosic polysaccharides and cellulose comprise the structural components of the cell wall (Hedley, 2001). Carbohydrates constitute 49.-68% of the dry weight of pulses. Starch is the most abundant carbohydrate in pulses (Arora, 1983; Chibbar, Ambigaipalan & Hoover, 2010).

For most pulses, starch accounts for 22-45% of the seed weight, depending on the species (Hedley, 2001; Hoover et al., 2010; McCrory et al., 2010). As in other grains and seeds, pulse starches are composed of amylose, a linear α-1,4-linked glucan with few branches and a molecular weight of 10^5–10^6, and amylopectin, a highly branched and much larger molecule with a molecular weight of 10^7-10^9 and composed of α-1,4-linked glycosyl units of varying lengths connected by α-1,6 branch points (McCrory et al., 2010). Structurally, amylopectin is divided into clusters containing the exterior branches and short linear chains, and an internal region comprising longer linear chains linking the clusters. Both the amylose long linear chain and the amylopectin external linear chains reassociate or "retrograde" on cooling following gelatinization; this retrogradation occurs much faster in amylose (McCrory et al., 2010).

Pulse starches generally have a higher content of amylose, ranging from 32-35%, compared to cereal and tuber starches; this factor, in addition to their associated high capacity for retrogradation, reduces the starch digestion rate, rendering them either slowly digestible or resistant to digestion and thus contributing to a low glycemic index (McCrory et al., 2010).

Amylose may sometimes occur as a major fraction of the starch in pulses, ranging from 24% to 88% (Ratnayake et al., 2001). Comparison and accurate assessment of amylose concentration among and between pulse starches is difficult due to differences in growth location, physiological state of the seed, cultivar and methodology used for determination (Chibbar, Ambigaipalan & Hoover, 2010). Starch solubility, lipid binding and some other functional properties are contributed by amylose in starch. The solubility of starch granules is assumed to be influenced by amylopectin (Reddy et al., 1984). The anthrone method was used to determine the total available carbohydrate in chickpea and dry pea flour, and yielded values that ranged from

62.5 % to 65.7% of dry matter (Berrios et al., 2010). These authors also reported that the total available carbohydrate in chickpea flour and dry pea flour consisted essentially of starch, based on the findings of Sosulski, Garrant & Slinkard (1976) and Swanson (1990), who determined the values to be 59% starch for chickpea flour and 54% starch for dry pea flour.

In dry pulse seeds, sugars constitute only a small percentage of the total carbohydrate. The oligosaccharides of the raffinose family (raffinose, stachyose, verbascose and ajugose) account for a substantial percentage of the total sugars in pulses. The predominant oligosaccharide depends on the type of pulse. Verbascose is the main oligosaccharide in fababean, whereas stachyose is the major oligosaccharide in Great Northern bean, lentil, and smooth and wrinkled pea (Reddy et al., 1984).

2.1.1.2.1 Dietary fibre

Pulses contain a considerable amount of dietary fibre. Whole seeds contain a higher amount of dietary fibre than flour or splits due to the higher fibre content in the seed coat than in the cotyledon. The seed coat also contains a higher proportion of insoluble fibre than does the cotyledon (Tiwari & Singh, 2012; Salunkhe, Kadam & Chavan, 1985). Dietary fibre consists of plant cell wall constituents such as cellulose, hemicelluloses, pectic substances and lignin. Cellulose plays a significant role in the utilization of nutrients. Increasing the level of cellulose in the diet reduces the utilization of ingested protein (Tiwari & Singh, 2012; Salunkhe, Kadam & Chavan, 1985). Pulses with a small grain size are higher in total dietary fibre than those with large grain size, since they have a greater surface to volume ratio than large grains (Wang, Daun & Malcolmson, 2003). Lentil and pea are excellent sources of dietary fibre (24-30 %), whereas chickpea was reported to contain 17% of dietary fibre; dietary fibre values for fababean ranged from 15-30 % (Perez-Hidalgo, 1997; Hove, King & Hill, 1978).

2.1.1.3 Lipid

Lipids are a group of heterogeneous compounds which include fatty acids, mono-, di- and triacylglycerols, phospholipids, sterols, sterol esters, lipoproteins and glycolipids (Pattee et al., 1983). The lipid content of pulses is usually less than 2%, but may be as high as 21%. Lipid is mainly found in the embryonic axis. The total lipid content of pulses varies depending on variety, origin, location, climatic, seasonal and environmental conditions, and the type of soil on which

they are grown (Tiwari & Singh, 2012). The total lipid in pulses consists of numerous classes such as neutral lipids, phospholipids and glycolipids. Their proportion in the seed varies with the species and variety from 32-51%, 23-38% and 8-12%, respectively. In most pulses, neutral lipid is the principal class; however, phospholipids and glycolipids also are present in considerable amounts (Salunkhe, Sathe & Reddy, 1983; Tiwari & Singh, 2012).

The lipid of pea contains ten different neutral lipids, comprising triacylglycerols, free sterols and sterol esters, which are major constituents, and monoglycerides, diglycerides, free fatty acids, waxes and pigments (Miyazawa, Ito & Fungino, 1975). The major unsaturated fatty acids found in pea, bean, lentil and chickpea are oleic acid and linoleic acid (Grela & Gunter, 1995). Palmitic and stearic acid are the major saturated fatty acids found in fababean, whereas the major unsaturated fatty acids were oleic, linoleic and linolenic acids (Akpinar, Ali Akpinar & Türkoğlu, 2001).

2.1.1.4 Minerals and vitamins

Minerals and vitamins together comprise the principal micronutrients of pulses. Pulses are rich in minerals important for human health, including substantial amounts of calcium, copper, zinc, potassium, iron and magnesium. Potassium represents 25-30% of the total mineral content of pulses (Salunkhe, Kadam & Chavan, 1985). Phosphorous occurs mainly as phytic acid, which may have a deleterious effect on the absorption and utilization of calcium and other cations through the precipitation of insoluble salts in the stomach and duodenum. Pulses are good sources of iron and other nutrients (Shukla, Dixit & Arora, 1983). Selenium, a microelement associated with maintenance of the cytochrome P450 system, DNA repair, enzyme activation and immune system function, is present in most pulses. Pulses contain appreciable amounts of zinc as well. The mineral content of pulses ranges from 0.08-0.44% of calcium, 0.12-0.24% of magnesium, 0.3-0.45% of phosphorous and 0.9-1.7% of potassium. Whole grain pulses have higher mineral contents than do decorticated grains (Tiwari & Singh, 2012).

Pulses are good sources of thiamine, riboflavin and niacin. Carotene, on the other hand, is found in only minor amounts (0.01-0.12 %) (Salunkhe, Kadam & Chavan, 1985; Kandlakunta, Rajendran, & Thingnganing, 2008). Pulses (except winged bean and hyacinth bean) serve as excellent sources of folate, which is an essential nutrient and is believed to reduce the risk of neural tube defects. Beans also are a good source of thiamine and pantothenic acid. On average, 100 g of

cooked pulses provides approximately 23% of the nicotinic acid, 50% of the thiamine, 15% of the riboflavin, 20% of the vitamin B_6, 20% of the folate and 30% of the pantothenic acid requirements of an adult. Fat-soluble vitamins and vitamin C have been found to be scarce in pulses (Lam & Lumen, 2003; Tiwari & Singh, 2012). Pulses contain vitamin A and vitamin E, but contain little vitamin C, although it can be found in sprouted forms (Raatz, 2014).

2.1.1.5 Antinutritional factors

Antinutritional compounds are found in raw seed and flour. These molecules disrupt the digestion process when consumed by monogastric species, rendering the seed less digestible. The antinutritional compounds identified in pulse crops are classified into two categories – protein and non-protein antinutritional compounds (Roy, Boye & Simpson, 2010).

The antinutritional factors include hemagglutinins (lectins), enzyme inhibitors, phytates, polyphenols, flatulence factors, lathyrogens, cyanogenic compounds, estrogens, goitrogens, saponins, antivitamins and allergens (Salunkhe, Kadam & Chavan, 1985). Non-protein antinutritional compounds include alkaloids, phytic acid and phenolic compounds such as tannins and saponins. Protein antinutritional compounds commonly found in pulses include lectins (hemagglutinins), trypsin inhibitors, chymotrypsin inhibitors, anti-fungal peptides, and ribosome-inactivating proteins (Roy, Boye & Simpson, 2010).

Research provides evidence that these non-nutritive, bio-active compounds are considered to be anti-nutrients because of their ability to decrease protein digestibility and mineral bioavailability, but they also exhibit some health protective effects (Chung et al., 1998; Mathers, 2002). Phytic acid exhibits antioxidant activity and protects DNA from damage. Phenolic compounds have antioxidant and other important physiological and biological properties and have been found to be associated with a reduced risk of colon cancer. Saponins have a hypocholesterolemic effect and exhibit anti-cancer activity (Singh, 2012; Phillippy, 2003; Yeh & Yen, 2003). Amylase inhibitors may prove to be potentially therapeutic in treating diabetes. Studies show that lectins may have protective effects with respect to oxidative DNA damage and cancer chemoprevention. Phenolic compounds provide antioxidant activity and phytosterols have been found to lower serum cholesterol levels (Singh, 2012).

2.2 Concentrated protein products from soybean and pulses

Pulses, due to their substantial protein content, are good raw materials for the preparation of protein concentrates and isolates. The presence of antinutritional compounds may be a concern in the development of appropriate processing technology, most of which was first applied to soybean. Soybean products have been extensively used as nutritional and functional protein ingredients since 1960. Soybean accounts for over one-half of all of the oilseeds produced in the world. Its acceptance is due to several factors such as favourable agronomic characteristics, profitability to the farmer and processor, high quality protein meal, edible oil products, and ample supply at modest prices. A good combination of amino acids and fatty acids make the protein and oil appropriate for a variety of applications (Lusas & Rhee, 1995; Endres, 2001; Wolf, 1970).

Soybean protein products are categorized into three groups based on their protein contents, namely soy flours and grits (40-50% protein), soy protein concentrates (65-70% protein) and soy protein isolates (>90% protein). Soy flours and grits are the least refined and are usually prepared by grinding of soybean meal after removal of oil. Soybean flours and grits from which water and/or alcohol soluble constituents have been removed are termed protein concentrates. The most refined soy protein products from which fat, sugars, cotyledonary fibre and water-soluble compounds have been removed are called soy protein isolates (Lusas & Rhee, 1995; Endres, 2001).

Processed products from pulses can be classified into pulse protein flours, concentrates and isolates. Similar to soybean products, the basis of classification lies in increasing the protein contents of each of these products. Pulses may be milled into fine flours and subsequently air-classified. The process of air-classification separates the flour into starch-rich and protein-rich fractions. The concentrated protein-rich flour fractions generally contain 40-70% protein depending on factors such as cultivar, moisture, efficiency of separation of starch and protein fractions by the air-classifier, and the protein content of the starting pulse flour (Tyler & Panchuk 1982). The protein-rich flour obtained from air-classification may be further purified to the level of a protein concentrate, which must contain at least 65% protein, or into an isolate, where the protein content may be as high as 90%. Pea protein concentrates are most commonly prepared by dry methods, and wet processing methods are used for the preparation of isolates (Saarela, 2011).

2.2.1 Protein products from soybean

2.2.1.1 Soy flours and grits

Soy flours can be categorised as full-fat, defatted, low-fat, high-fat or lecithinated. The classification is based on the extent of fat/lipid removal from dehulled soybean. Their protein contents range from 40% in full-fat flours to approximately 54% in defatted flour, and they may vary in fat content, particle size and heat treatment due to variations in the processing methods employed. Full-fat soy flours are further classified as enzyme-active, toasted or extrusion-processed. Lipoxygenase activity in each of these types aids in its differentiation from the others. While enzyme-active flours are preferred for their lipoxygenase activity in certain products, the inverse is the case for toasted full-fat soy flours where limited lipoxygenase activity is preferred as it leads to a beany flavour. Lipoxygenase activity in extruded products is inactivated by dry heating (Lusas & Rhee, 1995).

For the production of enzyme-active, full-fat soy flours, cleaned whole soybean seed is tempered, cooked and dried to 10% moisture content. The cotyledons are then dehulled and milled. In the production of toasted, full-fat soy flours, soybean is steamed under slight pressure for 20-30 minutes to curtail lipoxygenase activity, cooled, dried, cracked, dehulled and milled to produce flour. Low-fat soy flour production employs solvent extraction of dehulled cotyledons, followed by desolventization, drying and milling. Lecithinated soy flours have 1-15% of lecithin added to defatted flakes in order to improve the dispersion properties of the flour (Lusas & Rhee, 1995; Endres, 2001).

The application of soy ingredients in industry is greatly dependent on their Nitrogen Solubility Index (NSI). Defatted soy flours are classified as white (NSI 85-90), cooked (NSI 20-60) or toasted (NSI<20). High NSI flours (>90) are used as white bread bleaching agents, starting materials for production of protein isolates and spun protein fibers, and fermentation aids. Flours with NSIs of 60-75 are used in the production of soy protein concentrates and in food systems to alter fat and water absorption. Flours with NSIs of 10-45 are used as nutritional extenders in processed meats, bakery mixes and baby foods, and in the production of hydrolyzed vegetable protein ingredients. Flakes or grits find application as nutritive meat extenders (Lusas & Rhee, 1995; Endres, 2001).

2.2.1.2 Soy protein concentrates

Interest in the production of protein concentrates from soybean stemmed from the need to increase protein concentration and improve flavour (Anonymous, 1992). The various methods used to produce protein concentrates from soybean differ in the means employed to immobilize the protein and leach/extract the solubles such as flatulence sugars, soluble nitrogenous matter, mineral matter and other constituents (Meyer, 1825; Lusas & Hernandez, 1997). The most commonly implemented procedures for the production of protein concentrates are extraction with aqueous-ethanol, acid leaching and hot-water leaching. Protein concentrates made from soybean are categorized as traditional concentrates (produced by aqueous-alcohol washing of defatted soy flour), texturized concentrates (extruded in high pressure-temperature systems with moisture contents in the range of 20-30%) and low-antigen soy protein concentrates (produced by aqueous-alcohol washing under specific conditions in order to reduce the levels of antinutritional factors (Endres, 2001).

Aqueous-alcohol washing involves the leaching of soluble materials such as fat, sugars and some proteins (Horan, 1974). In this process, soy flour is extracted with 60-80% aqueous-ethanol. The solvent containing soluble constituents is separated from the flour and evaporated. The solubles/molasses are concentrated and recovered, the alcohol is condensed, and the wet flakes are desolventized to remove alcohol and water. Extracted concentrates are exposed to reasonably high temperature conditions and mechanical working, which improves solubility and functionality. Extraction of flour with aqueous-alcohol removes objectionable flavour and colour components. The protein concentrate obtained from ethanol-extracted flour is superior in colour and flavour with markedly improved foaming properties as a result of removal of phospholipids and other alcohol-soluble materials (Hua et al., 2005; Eldridge et al., 1963; Uzzan, 1988; Baker et al., 1979). Produced by this method, the concentrate suffers a high loss of isoflavones, and protein products produced with this process may have low NSIs due to denaturation by the solvent (Uzzan, 1998; Meyer, 1825; Johnson, 1976).

The acid leaching process operates on the principle that globulins are the dominant proteins in soybean and are insoluble at the isoelectric pH of 4.5. Defatted soy flakes are washed with water at pH 4-5, which removes mainly non-protein constituents such as soluble sugars from the protein-polysaccharide matrix, while most proteins (mainly globulins), polysaccharides and some minerals remain in the insoluble matter. Centrifugation is used to concentrate the solids to 20% followed by

neutralization with a food grade alkali and drying. Acid washing results in a greater loss of nitrogen as several minor proteins are soluble at the isoelectric pH (Uzzan, 1988; Meyer, 1825; Ohren, 1981; Tiwari & Singh, 2012).

In hot water leaching, soy flour is developed into a dough, following which the proteins are denatured under heat and pressure, usually by extrusion. The extruded product is leached with hot water, which yields a protein concentrate consisting of the proteins rendered insoluble by heating (Lusas & Rhee, 1995). This process results in dark coloured concentrates due to the application of heat (Ohren, 1981).

2.2.1.3 Soy protein isolates

Soy isolates contain at least 90% protein on a moisture-free basis. The soybean industry applies several processing methods in the manufacture of protein isolates, including alkaline extraction and isoelectric precipitation, membrane processing, aqueous extraction processing, and salt extraction (Lusas & Rhee, 1995; Endres, 2001).

In alkaline extraction and isoelectric precipitation, a mixture of ground flakes and water at ratios ranging from 1:5 to 1:20 is prepared. The mixture is adjusted to a pH of 8-11 using dilute sodium hydroxide and allowed to stand for 30-180 min in order to maximize the solubilization of protein. Elevated temperatures (55-65°C) may be used to further increase protein solubilization and extraction. Insoluble material is removed by filtration or centrifugation and the pH of the extract is brought to the isoelectric point (pH 4-5) by the addition of hydrochloric acid or other food-grade acid, which leads to precipitation of protein in form of a protein curd. The extract is then centrifuged to recover the protein precipitate. The precipitate is washed to improve its quality and purity and to facilitate removal of salts, neutralized to a pH of 6.8 with sodium or calcium hydroxide, and dried. Isolates prepared by alkaline extraction and isoelectric precipitation have variable protein contents, assumed to be due to differences in processing conditions (Uzzan, 1988; Boye, Zare & Pletch, 2010; Moure et al., 2006; Lusas & Rhee, 1995).

Ultrafiltration is a pressure-activated separation process which employs membranes to separate constituents in a liquid mixture (Tiwari & Singh, 2012). In this process, flour or meal is extracted with water at alkaline or acidic pH. The process temperature depends on the starting material and typically ranges from 43-55°C. The material is then centrifuged to remove fibre and then ultrafiltered to concentrate the proteins. Factors that affect the efficiency of separation include

the type of membrane, ultrafiltration conditions, the volume concentration ratio desired and the molecular weight cut-off (Boye, Zare, & Pletch, 2010; Lusas & Rhee, 1995). Isolates manufactured using ultrafiltration may have superior properties as compared to more conventional products prepared by isoelectric precipitation (Alibhai et al., 2006).

Aqueous extraction processing may be employed to remove oil from soybean, using water rather than an organic solvent, and to prepare a concentrate or isolate concurrently. In this process, soybean seed is dried to a moisture content of 6%, dehulled and milled. A slurry having a solids to water ratio of 1:12 (w/v) is prepared. It is adjusted to a pH of 9 at 60°C and 0.01% hydrogen peroxide is added to inactivate lipoxygenase. The slurry is centrifuged to effect separation of the aqueous, solid and oil phases. The aqueous phase is adjusted to acidic pH by the addition of HCl for precipitation of protein, which is recovered by centrifugation. The precipitate is then washed and dried (Lusas & Rhee, 1995).

Micellization, alternatively referred to as salt extraction, is used to separate and recover proteins by salting in and salting out. Flour is extracted with salt at an appropriate ionic strength followed by concentration of the extract. The solution is then diluted or dialyzed to cause precipitation of protein, which when dried becomes the isolate (Boye, Zare & Pletch, 2010; Lusas & Rhee, 1995). The solubility of protein in the solution in this process greatly depends on the concentration of salt in the solution (Tiwari & Singh, 2012).

2.3.1 Protein products from pulses

2.3.1.1 Dry fractionation/air-classification of pulse flours

Dry fractionation by fine milling followed by air classification is a more sustainable alternative to wet fractionation for production of protein concentrates from pea and other pulses (Pelgrom et al., 2013; Bergthaller et al., 2001; Emami, Tabil, Tyler & Crerar, 2002). The air classification process separates finely milled flour into starch and protein fractions, mainly on the basis of their aerodynamic properties, which result from a combination of density and particle size. Fine milling detaches the larger starch granules (>20 μm) from the smaller protein-rich particles (1-3 μm), which allows then separation (Pelgrom et al., 2013; Tyler & Panchuk, 1982). The composition of the seed plays a significant role in determining the amount and composition of the processed products produced from pulses by air classification and the distribution of fat, ash, fibre and non-starch carbohydrate between the fractions impacts the purity of the separated fractions

(Youngs, 1975). This process is not suitable for soybean due to its high fat and low starch content, which leads to poor efficiency in separation.

Coarsely milled flour fractions lead to the presence of aggregates of protein bodies, starch granules and other cell components, which hinders separation as some of the protein remains adherent to the starch granules after milling, thus affecting the yield of the protein fraction and the purity of the starch fraction (Pelgrom et al., 2013; Vose, 1978; Shapiro & Galperin, 2005). Conversely, very fine milling leads to extensive starch damage which affects separation negatively, as the starch granule fragments and protein bodies have similar sizes.

An air classifier is fundamentally an elutriator employing an air stream to separate a mixture of finer particles from coarser ones (Tyler, 1984). Whole or dehulled seed is pulverized into a very fine flour, followed by air classification in a spiral air stream to separate starch from protein (Boye, Zare & Pletch, 2010). Tyler (1982) described the process of separation of starch and protein fractions using an air classifier (Figure 2.1).

During air classification, the smaller protein-rich particles are separated from the larger starch granules. The protein content of the fine fraction is dependent on the protein content of the pulse flour, the dispersibility of the powder mixture in the air and the cut-point of the air-classifier (Pelgrom et al., 2013; Dijkink et al., 2007; Reichtert, 1982). Cut-point is the size at which a particle has a 50% probability to enter either the fine or the coarse fraction. It can be controlled by selection of air classification conditions, such as the classifier wheel speed and the air-flow (Pelgrom et al., 2013; Cloutt, Walker, & Pike, 1986). A cut-size of approximately 10 μm is optimum for efficient separation of protein and starch as it is just below the size of most starch granules. Dispersability is negatively influenced by particle agglomeration (Dijkink et al., 2007).

The process can be repeated in order to improve separation. However, this negatively affects the purity of separated constituents as more damaged starch will be present in the second protein fraction (Tyler, Youngs, & Sosulski, 1981). Air classification of pea flour containing 21% protein yielded 25% of fines with a protein content of 60% and a coarse fraction containing about 8% protein. The protein content of fababean protein after the first air classification step ranged from 71-75%. Remilling the starch fraction from fababean yielded a second-high protein fraction

containing 64-68% protein. Great Northern bean was reported to have 50% protein in the first fine fraction, and 41% protein in the fine fraction obtained after remilling (Youngs, 1975).

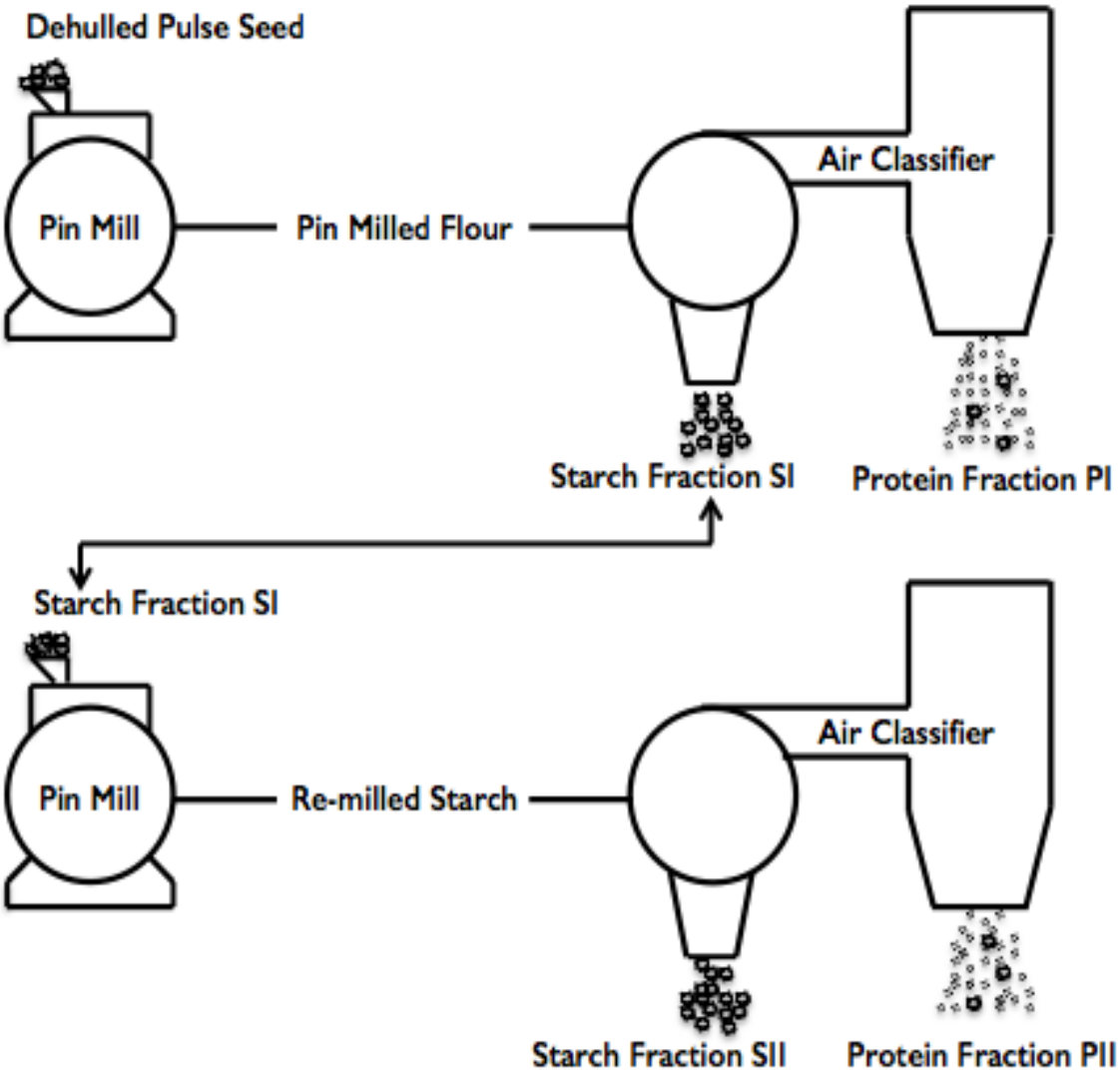

Figure 2.1: The double pass pin milling and air classification process (Tyler, 1982).

2.3.1.2 Pulse protein concentrates

The procedures described earlier such as extraction with aqueous-ethanol, acid leaching and hot-water leaching for the production of protein concentrates from soybean may be adapted for the preparation of protein concentrates from pulses. Pokatong (1994) prepared protein concentrates from air-classified pea protein and soy flour by aqueous-ethanol washing, acid (isoelectric) washing and alkaline extraction. Protein concentrates could also be prepared by using an enzyme liquefaction process. During this process, the starch is liquefied using alpha-amylase that breaks the alpha-1,4 linkages, thus leading to starch liquefication. The alpha amylase is then inactivated by heating and the degraded starch is washed out. This procedure does not involve the use of chemicals and hence the concentrates produced are considered safe for use. This method was reported by Paraman et al. (2006) for preparation of concentrates from rice; however, this is still to be demonstrated with pulses such as pea (Tiwari & Singh, 2012).

2.3.1.3 Wet separation of starch and protein fractions from pulses

Wet fractionation is a commonly used process for separation of starch and protein constituents of pulses. This process is used in the production of both concentrates and isolates. In wet fractionation, the starting material is reduced in size by milling and subsequently diluted to achieve disentanglement of the tissue structures to enable extraction of components such as protein, starch and lipids (Schutyser & Van der Goot, 2011). Youngs (1975) described the production of protein concentrates from field pea using a wet process (Figure 2.2). Ripe, yellow peas, whole or dehulled, were ground to a fine flour in a pin mill and the flour was slurried with five parts of water. This enabled the proteins to become dispersed and starch granules to become suspended. The pH of the slurry was increased to 9 by the addition of lime. The slurry was centrifuged to separate a protein-rich supernatant and a starch-rich solids fraction. The high-protein supernatant was spray or drum dried to yield a concentrated protein product (60% protein). The starch fraction, containing about 6% protein, was reslurried with five parts of water and centrifuged to produce a starch solids fraction containing about 2% protein. The subsequent batch of flour was slurried using wash water from the second extraction. A forced air oven at 60°C was used to dry the starch solids. A light yellow, essentially flavourless protein concentrate was obtained.

This process suffers some critical drawbacks as it uses copious amounts of water and energy; the mixing of flour and water and the drying of solids are the major energy consuming

steps during wet fractionation. Furthermore, the native functionality of the proteins is lost due to pH changes and elevated temperatures during dehydration (Pelgrom et al., 2015; Schutyser & Van der Goot 2011; Boye, Zare, & Pletch, 2010). It also fails to include insoluble proteins in the isolate, which are highly aggregated proteins with specific functionality (Pelgrom et al., 2013).

Pokatong (1994) prepared protein concentrates from air-classified pea protein and soy flour by aqueous-ethanol washing, acid (isoelectric) washing and alkaline extraction. For aqueous-ethanol washing, alcohol concentrations ranging from 50-95% were used. With the exception of 90-95% aqueous-ethanol-washed soy and pea products, all products qualified as true protein concentrates, i.e. protein concentrations were >65% on a dry-weight basis. Protein concentrates produced by 50% aqueous-ethanol washing were found to have the highest protein contents. It also was observed that as the concentration of the alcohol in aqueous-alcohol washing increased, there was a notable increase in product yield and protein recovery, but a decrease in protein content. Acid-washed concentrates were found to be free of oligosaccharides, whereas ethanol-washed products had significant residual levels. Alkali-extracted products contained lower amounts of raffinose and verbascose, but were enriched in sucrose. All of the methods yielded concentrates with reduced trypsin inhibitor activities. Acid-washed and alkali-washed products had lower nitrogen solubility indices (NSIs) compared to the starting material. Alcohol treatments at all concentrations had a negative impact on NSI. The acid-washed concentrates exhibited higher water hydration capacities (WHCs) compared to the alkali-extracted products, and products extracted at higher alcohol concentrations showed reduced WHCs. All products had comparable oil absorption values. However, the oil emulsification capacities of the acid-washed and alkali-extracted products were higher than those of the alcohol-washed products. The emulsifying activities of acid-washed and alkali-extracted products were found to be higher than those of the alcohol-washed products. The acid-washed pea concentrate had a significantly higher foaming capacity compared to the other products, but the lowest foam stability. Both foaming capacity and foam stability increased with an increase in alcohol concentration.

The performance of selected protein concentrates was tested in a high-fat meat emulsion system (Pokatong, 1994). Addition of 70%-ethanol-washed products to a bologna formulation resulted in an unstable emulsion with loss of fat and aqueous liquid during cooking. However, the products containing the protein concentrates were more stable than the controls.

2.3.1.4 Pulse protein isolates

Methods such as alkaline extraction and isoelectric precipitation, membrane processing, aqueous extraction processing, and salt extraction described in section 2.2.1.3 are common methods for production of protein isolates from pulses as well. The dominant fractions of globulins and albumins in the isolate produced are greatly affected by the processing method. Isolates prepared from isoelectric precipitation are mostly composed of globulins whereas salt extraction yields a mixture of albumin and globulin fractions (Stone et al., 2015; Kiosseoglou & Paraskevopoulou, 2011 and Liu et al., 2008).

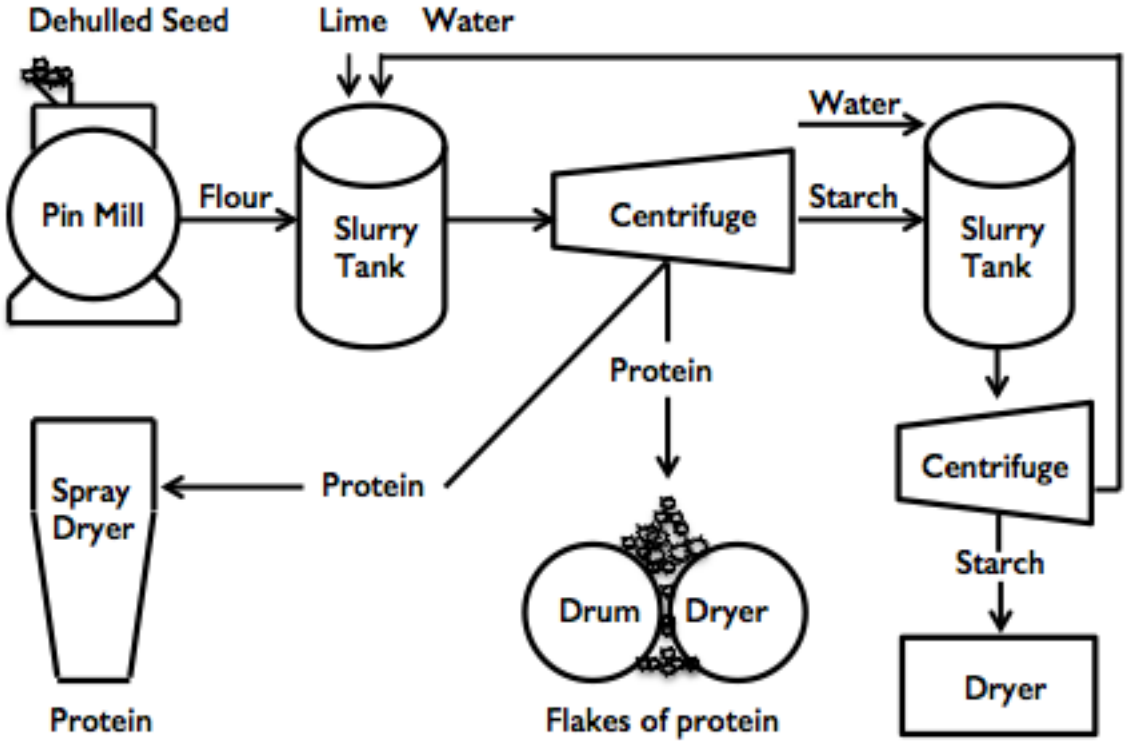

Figure 2. 2: Wet processing of pulses (Youngs, 1975).

2.3.1.5 Commercially available concentrated protein products from pulses

Recent advances in technology and the quest to find alternatives for meat proteins have put pulse-based protein products in the forefront. Soy protein has been leading the industry for several years. However, due to the GMO status and allergenicity problems associated with soy, pea protein is becoming a popular alternative. To address the growing demand for plant proteins. Roquette, has announced a notable investment of more than 400 million CAD to build a brand-new pea-protein manufacturing site in Portage la Prairie, Manitoba (Canada). Several commercial products are now available in the marketplace. Some leading names involved in the manufacture of pea protein products include Nutri-Pea Limited, Burcon, P&H Milling and Cosucra.

Nutri-Pea's pea protein line offers product designers an easy means of vegetable protein enrichment. Their marketed products include Propulse™, Propulse-N™ and Propulse-S™. Propulse™ is a natural, food-grade pea protein isolate offering high functionality and nutrition. Its applications include foods and beverages where protein enrichment is required. It has a bland taste and neutral flavour, and provides a surplus of amino acids. Propulse N™ is a natural food-grade pea protein primarily designed to meet requirements of beverage manufacturers. It has a high level of functionality and nutrition with a superior amino acid profile, especially lysine, is bland to the taste and has no effect on colour or flavor (Anonymous, 2016).

Burcon is another brand name that markets pea protein products. Their pea protein isolate is sold under the trade name Peazazz®. Peazazz® has clean flavour characteristics, is 100% soluble, transparent and heat stable in low pH solutions and is well-suited for use over a range of pHs Anonymous, 2016).

The P&H Milling Group manufactures several pea protein products including Prestige and Progress, using fine milling and air-classification.

Cosucra has a line of pea protein products branded as "Pisane", a unique non-GMO and gluten-free ingredient extracted by a natural process from the yellow pea (Anonymous, 2016).

2.4 Functional Properties

Functional properties are attributes, which at the proper concentration of the individual components and under appropriate conditions, provide desirable characteristics to the product (Sikorski, 2007). These properties of proteins affect the processing and behaviour of protein in food systems and can be observed in interactions with the surrounding solvent, ions, other proteins, saccharides, lipids and numerous other components, as well as in surface phenomena. Functional properties affect the appearance, colour, juiciness, mouthfeel and texture of a large variety of foods, as well as cutting, mincing, mixing, formation of dough, fibres and bubbles, shaping, and transporting of food materials (Sikorski, 2007; Kinsella, 1976). Pomeranz (1991) classified functional properties of proteins into groups namely a) sensory and kinaesthetic properties comprising flavour, colour, odour and texture, b) hydration, dispersibility, solubility and swelling properties that define protein-water interactions, c) surface active properties that include emulsification, foaming and absorption, such as fat binding, d) rheological properties including gelation and texturization, and e) properties such as adhesiveness, cohesiveness, dough making and film and fibre making. Hydration and fat absorption affect other properties, such as foaming and emulsification properties. Foam is formed as a result of air bubbles being entrapped by a surfactant, like proteins, in an aqueous phase (Kaur & Singh, 2007a; Kinsella, 1981), whereas emulsions are formed when oil droplets are dispersed in an aqueous medium with the help of a surfactant (Han & Khan, 1990a; Yin et al., 2010; Zhang et al., 2009; Toews & Wang, 2013).

The functional properties of a food component can be modified using enzymatic physical and chemical processes that change the structure of the proteins. They also rely on the pH, ionic strength and temperature of the food system. By better understanding the tertiary structure of many food proteins, it also should be possible to modify their functionality using genetic engineering. To evaluate the functional properties of some proteins in different systems, the quantitative structure-activity relationship approach may be applied (Nakai & Li-Chan, 1988).

2.4.1 Solubility

The solubility or extractability of proteins is often defined in food chemistry as the percentage of the total quantity of protein contained in the food material that can be extracted by water or a suitable solvent under specified conditions (Tiwari & Singh, 2012). Solubility depends on the properties of the protein surface (polar/non-polar amino acids) and the solvent, isoelectric

point, pH, concentration and charge of other ions, ratio of sample weight to solvent volume, particle size of the sample, duration of extraction, and temperature (Sikorski, 2007; Kiosseoglou & Paraskevopoulou, 2011). Protein-solvent interactions promote hydration and solubility (Nakai & Modler, 1996). Hall (1996) and Tiwari & Singh (2012) attributed solubility primarily to the proportion and distribution of hydrophilic and hydrophobic groups on the surface of the protein molecule. Generally, proteins rich in ionizable residues of low surface hydrophobicity are soluble in water or dilute salt solutions, as in these proteins the hydrophobic residues are buried in the interiors of the protein. Proteins abundant in hydrophobic groups readily dissolve in organic solvents, while showing low solubility in water as the hydrophobic residues create hydrophobic patches on the surface of the protein, thus negatively influencing solubility (Damodaran et al., 2008; Sikorski, 2007).

At isoelectric pH, protein solubility is markedly decreased as it carries a zero net charge, thus minimizing electrostatic repulsive forces. Under these conditions, hydrophobic interactions between bordering proteins can lead to aggregation and precipitation (Hall, 1996). At pHs above and below the pI, solubility is increased due to electrostatic repulsion brought on by positive and negative net charges on the protein surface. The solubility of most pulse proteins is highest at acidic and alkaline pH values (Hall 1996).

Solubility is a critical functional property as it affects the textural, colour and sensory properties of products (Meng & Ma, 2002), including emulsifying, foaming and gel forming properties (Shand et al., 2007). McWatters & Holmes (1979) measured the nitrogen solubility of soy flour over a pH range of 2.0-10.0 in three dispersion media, including water, 0.l M NaCl (low salt) and l.0 M NaCl (high salt). The primary determinant of the nitrogen solubility of soy flour was pH. It was found that the nitrogen solubility in water and low-salt suspensions was similar and was low at pH 4.0, but improved significantly at pHs below or above 4.0. In high-salt suspensions, nitrogen solubility generally increased from pH 2.0 to pH 10.0. Changing the electrovalent properties of the protein, either by changing pH or by modifying salt concentration, appeared to be the most significant factor influencing solubility (McWatters & Holmes, 1979; Smith & Circle, 1938).

Reinkensmeier et al. (2015) compared the solubility properties of pea proteins from whole pea flour (PF), air-classified pea protein fraction (PPF), pea flour made from hulls (PTF) and pea protein isolate (PPI). The pH of the extraction solution was adjusted from pH 1.0 to 12.0 and was then mixed with PF, PPF, PTF, or PPI. After a 30-min extraction, the pHs of the protein extracts were determined. The results revealed that the highest solubility was at pH 11.0-12.0 for all fractions after the pH was adjusted, and the protein solubility was lowest at pH 4-6 in the protein extract from PF and PPF, which corresponds to a pH value of 2.0 or 3.0, respectively. For PPI, the highest protein solubility was found to be at pH 12.0. Boye et al. (2010) reported that the lowest solubility of pea protein isolates was at pH values between 4.0 and 6.0. Sosulski & McCurdy (1987) investigated the protein solubility of pea flour, air-classified pea protein and pea protein isolate. They concluded that the proteins in pea flour were 80% soluble at pH 6.6, whereas the protein in air-classified protein exhibited a lower solubility of only 65%. The field pea isolates exhibited the lowest NSI value (38.1%) at pH 6.6. It also was found that at pHs above or below the isoelectric point, pea flour and air-classified pea protein exhibited high nitrogen solubility values. The overall poor solubility of the isolate was attributed to the effect of the extracting conditions employed in production. Fernández-Quintela et al. (1997) also reported lower solubility for pea protein isolate over a pH range of 4.0 to 6.0.

2.4.2 Water Holding Capacity

The ability to retain water against gravity is known as water holding capacity (WHC), and includes bound water, hydrodynamic water, capillary water and physically entrapped water (Moure et al., 2006). The ability of many foods to retain water is affected by the involvement of proteins in different structures. The terms water hydration capacity, water binding capacity, water holding capacity, water absorption and swelling have been used interchangeably and they describe the maximum amount of water a protein material can take up or the determination of total water absorbed. The WHC of proteins varies with the availability of polar amino acids, which serve as primary sites of water interaction with protein (Quinn & Palton, 1979; Kinsella, 1985; Tiwari & Singh, 2012). Hydration of protein is affected by the presence of polysaccharides, lipids and salts, by the pH of the food, and by the processing applied and storage conditions (Pomeranz, 1991).

In totality, the water-binding capacity of proteins is a function affected by several factors such as size, shape, steric factors, conformational characteristics, hydrophilic-hydrophobic balance

of amino acids in the protein molecules, the lipids, carbohydrates and tannins associated with proteins, the thermodynamic properties of the system (interfacial tension, energy of bonding), the physicochemical environment (pH, ionic strength, vapour pressure, temperature, presence or absence of surfactants), and the solubility of protein molecules (Chavan, McKenzie & Shahidi, 2001; Chou & Morr, 1979). However, the polar amino groups of protein molecules are the primary sites of protein-water interactions. Cationic, anionic and non-ionic sites bind different amounts of water (Kuntz, 1971).

Heywood et al. (2002) investigated the functional properties of low-fat soy flour (LFSF) of three categories produced on the basis of protein dispersibility index/residual oil (PDI/RO) groupings: low LFSF, 14.0 ± 5.0/6.5 ± 0; mid LFSF, 42.0 ± 3.0/7.4 ± 2; and high LFSF, 67.0 ± 4.0/10.4 ± 1, and compared these to a commercially available defatted soy flour (DFSF). The WHC values of the flours were low-6.75, mid-6.19 and, high-4.79, and that of DFSF was 6.70. Although the PDI readings of the high LFSF flours and DFSF were similar, there was a significant difference in the WHC value. The authors attributed the result to the amount of RO present in the high LFSF sample (11.0%) which was much higher than in the DFSF (< 0.5%). They concluded that the presence of this additional fat could result in fewer available hydrophilic binding sites for water holding by the protein.

Pelgrom et al. (2013) studied the functionality of impact-milled and air-classified pea flours. It was concluded that the behaviour of air-classified pea flour in water after centrifugation was dependent on the protein content of the flour, as samples with a higher protein content consisted primarily of smaller particles as well as a larger fraction of small starch fragments, that together contributed to a higher WHC. In order to evaluate the impact of denaturation on WHC, pea flours with different protein contents were heated. It was found that the concentrates that had the lowest protein contents had the highest WHCs. This effect was explained to result from the greater hydration capacity of pea starch in comparison to pea protein (Damodaran, 2008; Horvath et al., 1989; Sosulski & Youngs, 1979). Heating resulted in denaturation of protein and the gelatinization of starch, which consequently increased the WHC significantly compared to that of the non-heated pea flour (Pelgrom et al., 2013; Abbey & Ibeh, 1988; Owusu-Ansah & McCurdy, 1991). Pelgrom et al. (2013) described the behaviour in terms of the dissociation of the pea protein subunits upon heating, which provided more water binding sites. The positive effect of residual

starch on WHC was explained by Damodaran (2008), Horvath et al. (1989) and Sosulski & Youngs (1979).

Sumner (1981) developed laboratory-and pilot-scale processes for preparing pea protein isolate as sodium proteinate (PNaI) and isoelectric (PI) products. Water absorption was affected by extraction method, with salt-extracted isolates having higher WHCs than isoelectric protein isolates. It also was suggested that water absorption and nitrogen solubility were related, in that increasing denaturation by agents such as heat decreases NSI, with a corresponding increase in water absorption, to a point, followed by a decrease with further reduction in the NSI (Wu and Inglett, 1974).

Stone et al. (2015) investigated the physicochemical and functional properties of pea protein isolates derived from cultivars of three market class of pea (CDC Meadow, CDC Striker and CDC Dakota) using three different extraction methods, namely alkali-extraction and isoelectric precipitation, salt extraction-dialysis, and micellar precipitation. Isolates prepared by micellar precipitation yielded the highest WHCs (3.2-3.6 g/g), followed by alkali extraction-isoelectric precipitation (2.4-2.6 g/g) and salt extraction-dialysis (0.34-2.6 g/g). The higher WHC of isolates prepared by micellar precipitation were attributed to the greater hydrogen bonding with water by the side chains of polar groups exposed on the protein prepared by the micellar method. It was found that the effect of cultivar was different for each extraction method. All isolates prepared by alkali extraction-isoelectric precipitation had similar WHCs (2.4-2.6 g/g), whereas for isolates prepared by salt extraction-dialysis, CDC Meadow had the highest WHC (2.6 g/g), followed by CDC Dakota (1.5 g/g) and CDC Striker (0.3 g/g). The trend was different for micellar precipitation, with CDC Meadow exhibiting a lower WHC (3.2 g/g) than CDC Striker and CDC Dakota, (~3.5 g/g).

Water holding capacity also is deeply affected by cultivar and varies among pulses. Fernández-Quintela et al. (1997) reported that a fababean protein isolate with a protein content of 81% prepared using isoelectric precipitation had a water absorption capacity of 1.8 g/g, vs. 1.7 g/g for a pea protein isolate (84% protein) prepared using the same method.

2.4.3 Oil Absorption Capacity

Oil absorption capacity (OAC) is the ability of a material to absorb oil and is expressed as the weight of oil absorbed per gram of material (Boye, Zare & Pletch, 2010). It is an important functional property as it enhances mouth feel and helps to preserve flavour and stabilizes high-fat products and emulsions. The OAC is calculated as the percentage ratio of the fat absorbed by the sample and the weight of the sample. Variation in OAC is attributed to variations in the presence of non-polar side chains; OAC also is influenced by the source of protein (Tiwari & Singh, 2012). According to Lee & Lopez (1984), protein-lipid interactions are affected by protein conformation, protein-protein interactions and the spatial arrangement of the lipid phase arising as a result of lipid-lipid interactions.

The mechanism of oil absorption involves capillary interaction that permits the absorbed oil to be retained. Hydrophobic proteins play a key role in the absorption of oil. Sathe et al. (1982) reported that the OACs of legume flours were affected by particle size, protein and starch content, type of protein, and non-polar amino acid side chain ratios on the surface of the protein molecule (Chau et al., 1997). According to Kinsella (1976), proteins which are more hydrophobic show superior binding of lipid, indicating that non-polar amino acid side chains bind the paraffin chains of fats. Boye, Zare & Pletch (2010) suggested that the presence of non-polar amino acids enhanced oil absorption; in other words, higher OAC values were the result of a higher amount of available non-polar side chains in protein molecules (Du et al.,2014). This was confirmed by the difference in oil absorption values between native and defatted chickpea protein concentrates, as reported by Toews & Wang (2013), where the oil absorption of defatted Kabuli varieties was higher than that of the original sample due to the exposing of non-polar amino acids by the defatting process. Sosulski & McCurdy (1987) compared the chemical composition and functional properties of dehulled flours, air-classified protein fractions, and acid- or alkali-extracted protein isolates from field pea, soybean and fababean. The results indicated higher oil holding capacity values for pea protein isolates than for pea flours. Soybean flour exhibited higher OHC compared to field pea and fababean flours. The values for the flours resembled those of the air-classified protein fractions. The authors concluded that the protein compositions of the fractions were the principal determining factors of functionality.

Agboola et al. (2010) studied the functional properties of yellow field pea flour, protein isolate (Propulse), two high-fibre products (Centara III, Centara IV) and one high-fibre-starch ingredient (Uptake 80). Contrary to the study by Sosulski & McCurdy (1987), their results demonstrated that OHC values for the whole flour were significantly higher than values obtained for the other products. The protein isolate was found to have the lowest value for OHC, which was attributed to the lower levels of starch and fibre in the isolate when compared to the fibre products or the whole flour used in the study. The authors attributed the difference in results to the milling of flour from whole seed, including the hulls. As hulls are largely fibre, they absorb oil, thus producing higher OHC values.

2.4.4 Emulsifying Properties

An emulsion consists of two or more immiscible liquids such as oil and water, where one liquid is suspended as droplets in the other. Proteins help to form and stabilize emulsions by reducing interfacial tension and preventing coalescence by forming a physical barrier at the oil-water interface (Tiwari & Singh, 2012). The emulsifying properties of proteins are measured as emulsion capacity, emulsion activity index and emulsion stability index. Emulsion capacity is the ability of a protein solution or suspension to emulsify oil. Emulsion activity is the ability of protein to participate in emulsion formation and stabilize it. Emulsion stability index reflects the ability of protein to adsorb rapidly at the oil-water interface during formation of an emulsion, and prevent flocculation and coalescence by offering resistance to stress and changes at the interface. It is therefore related to the consistency of the interfacial area over a defined time period (Pearce & Kinsella, 1978; Subagio, 2006).

The emulsifying properties of proteins are dependent on several factors, such as the capacity of protein to decrease the interfacial energy due to adsorption at the oil-water interface, and the electrostatic, structural and mechanical energy barrier caused by the interfacial layer that opposes destabilization (Wagner & Gueguen, 1999). Emulsifying properties are affected by solubility, surface charge, hydrophobicity, protein source, processing conditions, presence of salts and extraction method (Tiwari & Singh, 2012). The decrease in the diameter of the droplets due to agitation increases the interfacial area exponentially.

Hutton & Campbell (1977) determined the emulsion stabilizing activity and thickening ability of a soy protein concentrate (Promosoy-100) and isolate (Promine-D) at pH 5.0, 6.0 and 7.0

and at 4°C, ambient temperature (22-25°C) and 90°C. Their aim was to investigate the combined effects of pH and temperature on emulsion properties and thickening functions, and of temperature on fat absorption. They found that emulsions prepared from the concentrate had less emulsified oil after centrifugation than those prepared from the isolate.

Horvath et al. (1989) studied the functional properties of air-classified yellow pea fractions and reported emulsifying activity and emulsion stability values for pea flour and air-classified protein. The emulsion activity of the air-classified fraction was higher than that of the flour (64% and 58%, respectively), whereas they exhibited similar emulsion stabilities of 73%. Can Karaca, Low & Nickerson (2011) compared the emulsifying and physicochemical properties of protein isolates from chickpea, fababean, lentil and pea prepared by isoelectric precipitation and salt extraction to those of a soy protein isolate. Both legume source and isolate production method had a significant impact on the physicochemical and emulsifying properties of the isolate. The pea isolate was found to have the lowest emulsion capacity and stability, which was attributed to its high surface hydrophobicity, low surface charge and low solubility. Isoelectric precipitation resulted in isolates with higher surface charge and solubility compared to those produced via salt extraction.

2.4.5 Foaming Capacity

Food foams are colloids of gas bubbles trapped in a continuous liquid or semi-solid phase. In simple terms, a foam consists of dispersed gas bubbles surrounded by a continuous phase (Campbell & Mougeot, 1999). Foaming is responsible for the desirable rheological properties of many foods, such as the texture of bread, cakes, whipped cream, ice cream and beer froth. Thus, foam stability may be an important food quality criterion. However, foams are often a hindrance for the food processor, such as in the production of potato starch or sugar, and in the generation of yeast (Tiwari & Singh, 2012; Sikorski, 2007).

Foaming capacity and foam stability depend on the ability of proteins to form an interfacial film which maintains the air bubbles in suspension and slows down the rate of coalescence. Foaming properties are reliant on proteins and other components, such as carbohydrates (Sreerama et al., 2012; Du et al., 2014).

Proteins are present in the continuous phase and at the interface, and they have the ability to interact with adjacently adsorbed proteins, giving rise to interfacial films with the properties of thickness, gas permeability, shear viscoelasticity and dilatational viscoelasticity (Foegeding & Davis, 2011; Dickinson, 1992; Dickinson, 1999; Wilde et al., 2004). Interfacial films have been investigated under two concentration systems- micromolar and millimolar protein concentrations. When the majority of the protein is at the interface with a minimal concentration remaining in the continuous phase, it is referred to as the micromolar or lower protein concentration range (Foegeding & Davis, 2011; Cornec et al., 1999; Paulsson & Dejmek, 1992; Wierenga et al., 2009). In contrast, where there is an excess amount of protein in the continuous phase, presenting the possibility of exchange with the interface, it is termed millimolar protein concentration (Nicorescu et al., 2011; Yang & Foegeding, 2010; Foegeding & Davis, 2011).

Foods that contain foam structures are evaluated in terms of three important and partially distinct stages: formation, stability and consumption. During foam formation, protein contributes by rapidly adsorbing at freshly formed air/water interfaces resulting in lower interfacial tension, and by altering the viscosity of the continuous phase. Once formed, proteins contribute to the prevention of the foam destabilization mechanisms such as creaming, flocculation, coalescence and disproportionation (Foegeding & Davis, 2011; Damodaran, 2005; Dickinson, 1992; Hilgenfeldt et al., 2001; Koehler et al., 2000; Martin et al., 2002; Murray, 2007; Rodríguez-Patino et al., 2008; Wilde, 2000).

Hua et al. (2005) prepared a soy protein isolate by washing defatted soy flakes with aqueous alcohol and then extracting with water (ASPI) and compared its functionality to that of a conventional soy protein isolate (CSPI). They found that foam stability was improved through alcohol washing and the foaming capacity of soy protein isolates increased by 25% as compared to the conventional SPI. They also found that ASPI could maintain a high foam volume, thus indicating good foam stability.

Boye et al. (2010) compared the functional properties of yellow pea, green lentil, red lentil and (Kabuli and Desi) chickpea protein concentrates prepared using IEP and UF techniques optimized for extraction of proteins. They found that foaming capacity ranged from 98% to 106% and was similar for pea and lentil protein concentrates irrespective of the extraction method used. Desi and Kabuli chickpea concentrates prepared using IEP had higher foaming capacity than

yellow pea or lentil (green or red). Chickpea had higher foaming capacity but lower foam stability as compared to all other pulses. Foam stability values also depended on the extraction method used. Concentrates prepared by the IEP method were found to have higher foam stability than concentrates prepared by the UF method.

3. MATERIALS AND METHODS

3.1 Air-classified pulse protein fractions

Air-classified pulse protein fractions for preparation of protein concentrates were supplied by Parrheim Foods, Saskatoon SK. The fractions were prepared from seed accessed in 2012 from producers in Saskatchewan (pea, lentil and fababean) or Alberta (navy bean). Chickpea and pea flours were purchased from Best Cooking Pulses Portage la Prairie, MB. Commercial soy protein concentrates (Arcon S and Arcon F) which were used as controls were provided by Archer Daniels Midland Co., Chicago, IL.

3.2 Aqueous-alcohol washing and solvent extraction

3.2.1 Statistical analysis

Response surface methodology was used to construct mathematical models using a Box Behnken design (King & Zall, 1992), making it possible to quantitatively describe the relationship between treatment combinations and the protein contents and yields of the pea protein concentrates. The data obtained for protein and yield were analyzed using SAS Version 9.3 Software (SAS, Cary, NC).

3.2.2 Methods of protein concentrate preparation

Aqueous-alcohol (ethanol or isopropanol) washing of air-classified pea protein was carried out using several combinations of aqueous-alcohol concentration, temperature and time to fit the limits of a Box Behnken Response Surface Methodology. A flour to solvent ratio of 1:5 (w/v) (200 g of air-classified pea protein slurried in 1000 mL of solvent) was employed. The mixture of aqueous-alcohol and pea protein was mixed using a magnetic stirrer for various time intervals and at varying temperatures and then centrifuged using an Avanti J-E centrifuge (Beckman Coulter, Brea, CA) at 2000 x *g* for 10 min at 4°C. The cake obtained from centrifugation was extracted two more times in aqueous-alcohol of the same concentration. The thrice-extracted sample was given a final wash with 95% aqueous-alcohol and desolventized in a fumehood for 1 h. The protein concentrate was then dispersion dried (Lab-Line High Speed Fluid Bed Dryer, Lab-Line Instruments, Melrose Park,

IL) at ~70°C, a blower speed setting of 7, and a drying time of 20 min. The same process was used for preparation of protein concentrates from air-classified lentil, fababean and bean protein.

3.2.3 Preparation of extracts for analysis

Extracts were obtained by aqueous-ethanol washing of air-classified pea protein at 52% aqueous-ethanol, 32°C and 12 min or at 65% aqueous-ethanol, 40°C and 11 min, deemed as optimal treatment combinations for protein content and concentrate yield, respectively, using Box Behnken Response Surface Methodology. Due to their resinous consistency, evaporated extracts posed difficulty in analysis hence calcium carbonate ($CaCO_3$) was added to the extract prior to evaporation. A 5% (w/v) dispersion was prepared by dispersing $CaCO_3$ in the total volume of extract obtained by aqueous-ethanol washing of air-classified pea protein. The extract was then dried to fine powder using a rotary evaporator (Buchi Rotavapor R-II Rotary Evaporator, Buchi Labortechnik AG, Flawil, Switzerland). The powder was then used for the analysis of composition and for gel electrophoresis

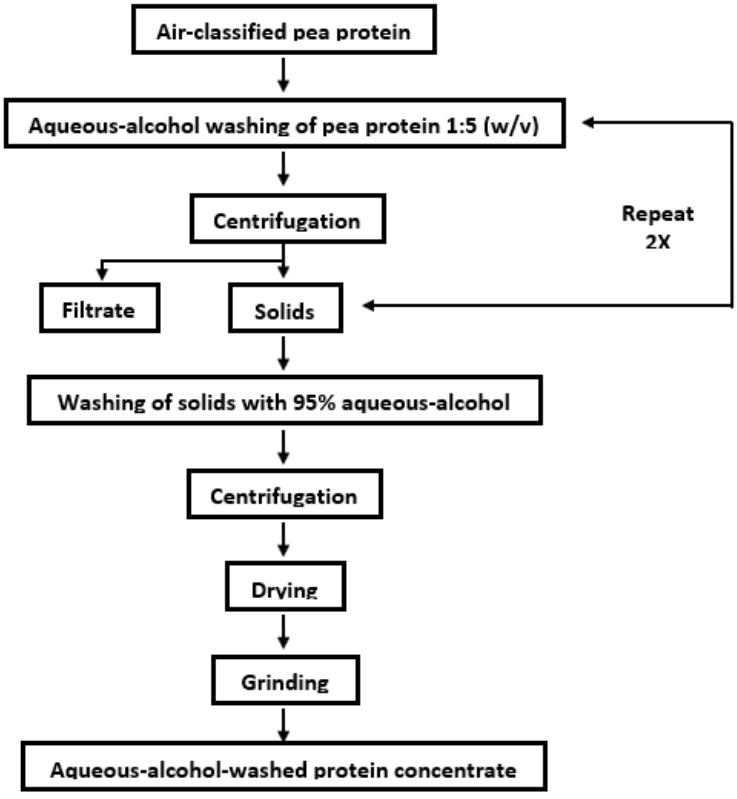

Figure 3.1 Aqueous-alcohol washing of air-classified pea protein.

3.2.4 Aqueous-alcohol washing/hexane washing of pea and chickpea flours

Extraction of crude fat from pea and chickpea flours was carried out using 70% aqueous-ethanol or hexane. A flour to solvent ratio of 1:5 (w/v) (200 g pea or chickpea flour was slurried in 1000 mL of solvent) was employed. The mixture of aqueous-alcohol or hexane and pea/chickpea flour was homogenized using a magnetic stirrer for 20 minutes at room temperature and then centrifuged using an Avanti J-E centrifuge (Beckman Coulter) at 2000 x *g* for 10 min at 4°C. The cake obtained from centrifugation was extracted two more times in 70% aqueous-ethanol or hexane. The 70% ethanol-extracted sample was given a final wash with 95% aqueous-alcohol and desolventized in a fumehood for 1 h, as was the hexane-extracted sample. The cake obtained was then dispersion dried (Lab-Line High Speed Fluid Bed Dryer, Lab-Line Instruments) at ~70°C, a blower speed setting of 7, and a drying time of 20 min.

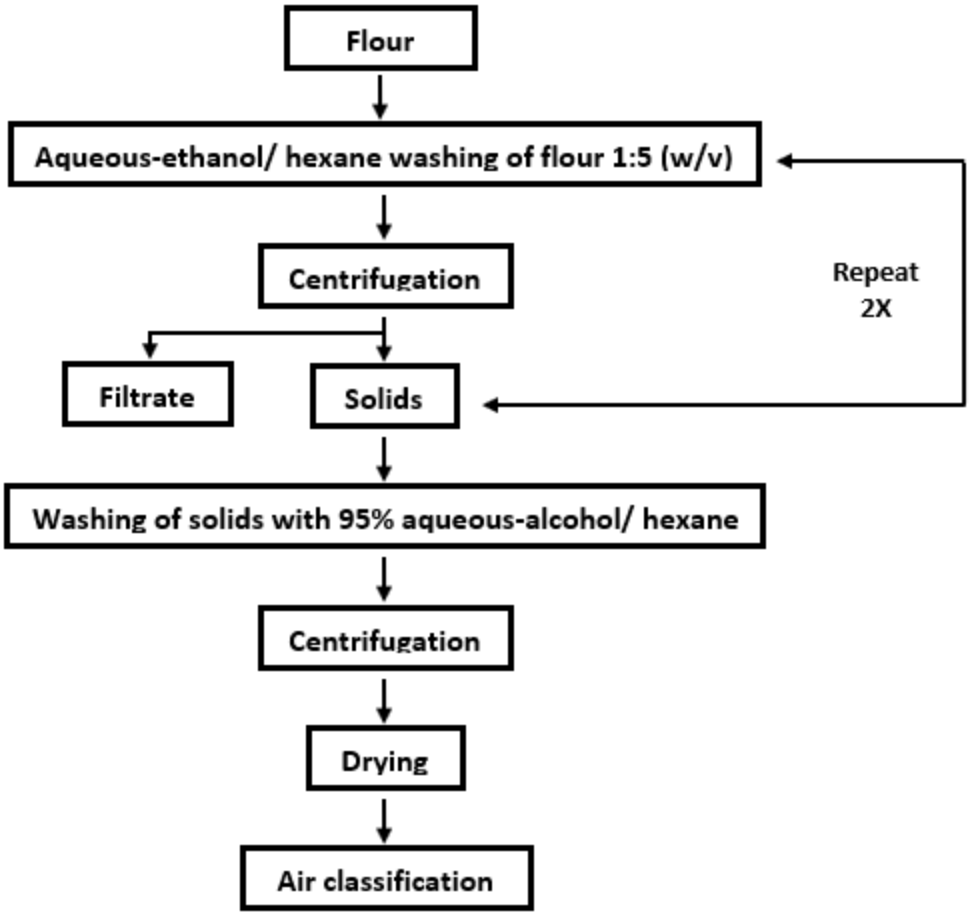

Figure 3.2. Aqueous-alcohol/hexane washing of pulse flours prior to air classification.

3.2.5 Fine milling and Air classification

Fine milling and air-classification of pea and chickpea flours was carried out at the Canadian International Grains Institute, Winnipeg MB. Five hundred grams of pre-milled flour was fed into a 100 UPZ Fine Impact Mill, (Hosokawa Alpine Aktiengesellschaft, Augsburg, Germany) operating at 20,000 rpm. The flour obtained was collected in a pre-weighed collection unit and the yield was determined. The pin-milled flour was then air-classified using a 100 MZR Hosokawa Alpine air-classifier (Hosokawa Alpine Aktiengesellschaft). A 100-g sample was weighed into the wide neck bottle and fitted to the feed device. The air-classifier speed was set at 10,000 rpm and the air-flow rate was set at 46 m^3/h to achieve a desired cut point. The air-classifier then separated the flour into starch and protein fractions, which were collected in the coarse and fine fraction bottles, respectively.

Figure 3.3. 100 UPZ Fine Impact Mill used for pin milling of pea and chickpea flours.

Figure 3.4. 100 MZR Hosokawa Alpine air-classifier used for air classification of pea and chickpea flours.

3.2.6 Mass balance and protein recovery

Alcohol-washed protein concentrates were prepared on a small scale (50 g of air-classified protein) to permit accurate determination of product yield and protein recovery.

3.3 Compositional analysis

The moisture, protein, starch, crude fat and ash contents of air-classified pulse protein fractions, aqueous-alcohol washed pulse concentrates, dried extracts and flours (solvent-washed and unwashed) were determined according to methods 44-19.01, 46-30.01, 76-13.01, 30-25.01 and 08-01.01 of the American Association of Cereal Chemists International (AACCI 2015). Total lipid content was determined by the method of Sahasrabudhe (1979). Oligosaccharide analysis was carried out by the method of Gangola et al. (2014). All measurements are reported as the mean ± one standard deviation (n = 2).

3.3.1 Moisture

Moisture contents were determined gravimetrically using a gravity-flow convection oven (Fisher Scientific™ Isotemp™ Standard Lab Oven, Thermo Fisher Scientific Inc., Waltham, MA). The oven temperature was set at 135°C. Three-gram samples were weighed into pre-dried and pre-weighed aluminum dishes and placed in the oven for 2 h. Moisture content (%) was calculated using the following equation:

$$\% \text{ Moisture} = \frac{\text{Loss of moisture x } 100}{\text{Weight of sample}}$$

3.3.2 Protein

The protein contents of samples were determined using a FP-528PC Protein/Nitrogen Analyzer (LECO Corp., St Joseph, MI). Approximately 0.24 g of sample was weighed into Quik-Cap Capsules (LECO) and combusted at 950°C in the presence of oxygen. Nitrogen freed upon pyrolysis was then separated from other products through a chromatographic column and quantified. A nitrogen-to-protein conversion factor of 6.25 was used.

3.3.3 Starch

Determination of total starch was carried out using a Total Starch Assay Kit (AA/AMG) (Megazyme International Ireland, Wicklow, Ireland). Briefly, 100 mg of sample was weighed into a glass test tube and 0.2 mL of aqueous ethanol (80%, v/v) was added. The tube was then vortexed (VWR Vortex Mixer, VWR International, Radnor, PA) for 10 sec and 3 mL of thermostable α-

amylase was added. The contents of the tube were then microwaved for 10 sec. Amyloglucosidase, 0.1 mL, was added to the tube followed by mixing (VWR Vortex Mixer) and placed in a water bath at 50°C for 30 min. After incubation, the contents of the test tube were transferred to a 100-mL volumetric flask and the volume was made up to 100 mL. An aliquot of this solution was transferred to a 15-mL centrifuge bottle and centrifuged at 3,000 rpm for 10 min. The clear, undiluted filtrate was transferred in duplicate aliquots (0.1 mL) to glass test tubes and 3.0 mL of GOPOD Reagent was added. Reagent blanks were prepared using 0.1 mL of water and 3.0 mL of GOPOD. The sample and blanks were then incubated at 50°C for 20 min and the absorbance for each sample was read at 510 nm against the reagent blank.

3.3.4 Crude fat

Crude fat contents were determined using a Goldfisch Fat & Oil Extractor (Labconco Co., Kansas City, MO)**.** Three-gram samples were weighed into pre-dried thimbles and extracted using petroleum ether for 8 h. The solvent was then recovered and the residual fat was allowed to dry in an air-oven at 100°C for 30 min, desiccated, cooled and weighed. Crude fat was calculated as:

$$\% \text{ Crude fat} = \frac{\text{Weight of extract-blank x } 100}{\text{Weight of sample}}$$

3.3.5 Total lipid

The modified method of Sahasrabudhe (1979) was used for determination of total lipid. Briefly, three-gram samples were weighed into pre-dried thimbles and extracted using choloroform-methanol (2:1, v/v) for 8 h using a Goldfisch Fat & Oil Extractor (Labconco). The chloroform-methanol mixture was recovered and the residue was dried in an oven at 100°C for 30 min, desiccated, cooled and weighed. Total lipid was calculated as:

$$\% \text{ Total lipid} = \frac{\text{Weight of extract-blank x } 100}{\text{Weight of sample}}$$

3.3.6 Ash

Ash was determined gravimetrically using a muffle furnace (Isotemp^{TM} Basic Muffle Furnace, Thermo Fisher Scientific, Inc.). Crucibles were pre-dried for 1 h at 550°C, cooled and dessicated. Samples of approximately 3 g were weighed into the pre-dried crucibles. The samples were then charred using a hot plate set at high heat in a fumehood until only black residue remained. The crucibles were then covered with lids and ashed overnight at 550°C. The white ash residue was then cooled and desiccated for 1 h. The ash content was measured as:

$$\% \text{ Ash} = \frac{\text{Weight of ash x 100}}{\text{Weight of sample}}$$

3.3.7 Oligosaccharides

The analysis was conducted according to the method of Gangola et al. (2014) in three phases of extraction, purification and sample preparation for HPLC.

3.3.7.1 Extraction of soluble sugars

According to modifications of methods by Frias et al. (1994), Sanchez-Mata et al. (1998) and Tahir et al. (2011), 500 mg of sample was weighed into a 15-mL falcon tube and 10 mL of 80% (v/v) ethanol was added to it. The contents of the tube were thoroughly stirred and the tube was mixed by vortexing. It was then incubated in a shaking water bath at 60°C for 45 min with intermittent mixing at 15-min intervals. The contents of the tube were then centrifuged using a Eppendorf Centrifuge 5804 R (Eppendorf AG, Hamburg, Germany) at 12,100 x *g* for 10 min. The supernatant was then collected in a separate tube and the pellet was re-extracted as described above. The extraction was repeated a third time and the supernatants were pooled and purified.

3.3.7.2 Purification of soluble sugars

A C-18 cartridge (Honeywell Burdick &Jackson, Muskegon, MI) was first washed with 5 mL of 99% methanol, followed by rinsing with 5 mL of distilled water and 3 mL of extract. The solution was discarded from the vacuum apparatus and glass tubes were placed on stands to collect the purified sample. Three milliliters of extract again was circulated through the column and a 1.6 mL aliquot of this extract was used for vacuum drying in a Speedvac® Vaccuum Concentrator (Thermo Fisher Scientific).

3.3.7.3 Sample preparation for HPLC

The vacuum dried samples were dissolved in 1000 µL of nano pure water with vortex mixing (VWR Vortex Mixer) followed by centrifugation at 10000 x *g* for 10 min. One hundred and twenty-five microlitres of clear upper aqueous phase was made to a final volume of 500 µL and the Raffinose Family Oligosaccharide profile was determined using a Waters HPLC-RI system (Waters Co., Milford, MA) controlled by Chromellon 7.0 software (Thermo Scientific).

3.4 Functional analysis

Air-classified protein fractions and aqueous-alcohol washed concentrates were assessed for several functionalities. Nitrogen solubility index (NSI) and water hydration capacity (WHC) were determined according to methods 46-23.01 and 56-30.01 of the AACCI (2015). For the determination of oil holding capacity (OHC), the modified method of Chakraborty (1986) was used. Emulsifying activity (EA) and emulsion stability (ES) were determined according to the method of Yasumatsu et al. (1972), and foaming capacity (FC) and foam stability (FS) were measured according to the modified method of Naczk et al. (1985). Colour was measured using a HunterLab spectrocolorimeter (Hunter Associates Laboratory, Reston, VA). All measurements are reported as the mean ± one standard deviation (n = 2).

3.4.1 Nitrogen solubility index

A 1-g sample was weighed into a beaker and 200 mL water at 30°C was added to it in small portions. The mixture was stirred at 120 rpm with a mechanical stirrer for 120 min. The contents were transferred into a 250-mL volumetric flask and the volume was made up to 250 mL. Two drops of antifoam was added to the mixture followed by thorough mixing. Forty milliliters of this solution was decanted into 50-mL centrifuge tube and centrifuged at 1500 rpm for 10 min and the supernatant was transferred to a 100-mL beaker. Twenty-five millilitres of supernatant was pipetted into a Kjeldahl flask and then analyzed for protein according to Method 46-16.01 of the AACCI (2015).

3.4.2 Water hydration capacity

A 5-gram sample was weighed into a pre-weighed 50-mL centrifuge tube and distilled water was added in small increments until the sample was completely wetted. The contents were centrifuged at 2000 x *g* for 10 min (Beckman Coulter Allegra centrifuge, Beckman Coulter,

Mississauga, ON).. The supernatant was discarded and the residue was weighed and the approximate Water Hydration Capacity was calculated as:

$$\text{Approximate WHC, mL/g} = \frac{(\text{Weight of tube+sediment})-(\text{Weight of tube+5.0})}{5}$$

Based on values obtained from the above equation, WHC was determined as:

$$\text{Weight of material} - \frac{15}{\text{approximate WHC}+1}$$

where 15 represents the desired total weight of sample and water.

The calculated weight of material was added to four centrifuge tubes, and to these water in volumes of 1.5 and 0.5 mL more, and 1.5 and 0.5 mL less, than that calculated (15 - weight of material) was added. The contents of each tube were thoroughly mixed and then centrifuged at 2000 x *g* for 10 min. Two tubes, one with and one without supernatant, represented the range in which the WHC value laid. WHC value was calculated as the midpoint between these two volumes, divided by the weight of the material.

3.4.3 Oil holding capacity

Oil holding capacity (OHC) was determined by weighing 1-g protein samples into 50-mL centrifuge tubes to which 10 mL of canola oil was added. The mixture was then vortexed for 10 sec every 5 min on maximum speed, for 30 min. Samples were centrifuged (Beckman Coulter Allegra centrifuge, Beckman Coulter) at 2000 x *g* for 10 min. The sample was then decanted and OHC was measured as:

$$\text{OHC (g/g)} = \frac{\text{Weight of wet sample-Weight of dry sample}}{\text{Weight of dry sample}}$$

3.4.4 Emulsion activity and stability

Emulsion activity and stability were measured by suspending 7 g of product in 100 mL of

distilled water to which a 100 mL of canola oil was added. The mixture was then homogenized using a Macro Homogenizer (Omni International, Marietta, GA) at 10,000 rpm for 1 min. The emulsion formed was then transferred into 50-mL centrifuge tubes and centrifuged using a benchtop centrifuge (Allegra X-22R, Beckman Coulter) at 1300 x *g* for 5 min. The height of the emulsified layer and total solution in the tube were measured using a lab ruler and emulsion activity was measured as:

$$\text{EA } \% = \frac{\text{Height of emulsified layer}}{\text{Total height of emulsion}} \text{x} 100$$

For emulsion stability, emulsions were prepared by the method used above, heated at 80°C for 30 min, cooled under tap water for 15 min, and centrifuged at 1300 x *g* for 5 min, and the emulsion stability was calculated as above.

3.4.5 Foaming capacity and foam stability

Fifty milliliters of a 3% (w/v) dispersion of sample and distilled water were homogenized using a Macro Homogenizer (Omni International) at 10,000 rpm. The mixture was immediately transferred into a 250-mL graduated cylinder and the foam volume recorded. Foam stability was expressed as the volume of foam remaining after 20, 40, 60 and 120 min. Foaming capacity and stability were calculated according to the following formula:

$$\text{FC} = \frac{\text{Volume of foam at 0 minute after homogenization}}{\text{Initial volume of the suspension}} \text{x} 100$$

$$\text{FS} = \frac{\text{Volume of foam at 20 to 120 minute after homogenization}}{\text{Initial volume of the suspension}} \text{x} 100$$

3.4.6 Colour analysis

The colour of samples was determined using a spectrophotometer (HunterLab MiniScan XE 45/0-L, Hunter Associates Laboratory, Inc., Reston, VA) The equipment was standardized using black and white tiles according to the method of Ubayasena et al. (2010). Colour was expressed in terms of L, a, and b values.

3.5 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was performed on extracts obtained by aqueous-ethanol washing of air-classified pea protein fraction at of 52% aqueous-ethanol, 32°C and 12 min and at 65% aqueous-ethanol, 40°C and 11 min, which were deemed as optimal treatment combinations for protein content and concentrate yields, respectively, using Box Behnken Response Surface Methodology according to Laemmli (1970). In brief, 75 µL of a 2 mg/mL powder-water suspension was diluted with 75 µL of water to give a final sample concentration of 0.1% (v/v). To all samples, 142.5 µL of Laemmli sample buffer (BIO-RAD, Mississauga, ON), and 7.5 µL of β-mercaptoethanol was added. The samples were then vortexed for 10 s, the vortexed samples were heated at 95°C for 5 min using an Incu Block model 285 (Denville Scientific Inc., South Plainfield, NJ, USA), and then centrifuged using an Eppendorf Centrifuge 5424 (Eppendorf, Hamburg, Germany) at 12,000 x *g* for 5 min. Pre-cast, 4-20% Precise Tris-glycine gels (Thermo Fisher Scientific, Waltman, MA) were used to run the samples in a MGV-202 Vertical Mini-Gel System (CBS Scientific, Delmar, CA) for a period of ~40 min at 200V using a Power Source 300V Electrophoresis Power Supply (VWR, Mississauga, ON). Precision Plus Protein™ Prestained Standards (BIO-RAD) ranging from 10 kDa to 250 kDa were used as molecular markers. Gels were then stained using Phastgel Blue R tablets (GE Healthcare Life Sciences, Uppsala, Sweden) for 1 h, followed by de-staining with methanol:water:acetic acid (3:6:1) (v/v/v) three times over a 24-h period. The de-stained gels were then scanned using an EPSON Perfection V750 Pro scanner (EPSON, Markham, ON).

4. RESULTS

4.1 Comparative study of the composition and functionality of protein concentrates prepared by aqueous-ethanol or aqueous-isopropanol washing of air-classified pea protein

4.1.1 Box Behnken Experimental Design

The primary objective of an optimization study is to maximize the output. This goal is achieved by developing an experimental set-up, changing one variable at a time. This process is difficult due to the constraints it lays down, such as a large experimental design, which involves multiple randomized treatment combinations that are difficult to achieve, cost limitations, and the effect of interactions among variables is not revealed. A Box Behnken statistical design with 3 factors, 3 levels and 13 treatment combinations with replicated centre-point treatments was used for this study. The design is constructed to resemble a multidimensional cube with a set of points lying at the mid-point of each edge and a replicated centre-point. The dependent and independent variables are listed in Table 4.1. The regression model fitted to the experimental results was as follows:

$$y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

where y is the dependent variable, β_o is the intercept, and β_1 to β_{23} are regression coefficients.

Table 4.1. Variables and their levels in the Box Behnken design.

Variables	**Levels**		
Independent Variables	**Low**	**Medium**	**High**
X_1= Alcohol concentration	50	60	70
X_2= Temperature	20	40	60
X_3= Time	5	10	15
Dependent Variables			
Y_1= Protein content			
Y_2= Yield			

4.1.1.3 Data Analysis

A Box Behnken statistical design with three factors – alcohol concentration, temperature and time – at three levels of each was used to study their effect on two dependent factors, the protein content and the yield of the protein concentrates. The results for aqueous-isopropanol-washed and aqueous-ethanol-washed concentrates showed that protein content tended to decrease as the concentration of alcohol increased, whereas yield tended to increase (Table 4.2). Time and temperature were found to have no significant effect on protein content or yield. The predicted models for the effects of alcohol concentration, extraction temperature and extraction time are presented in Figures 4.1 and 4.2.

Table 4.2. Box Behnken design with measured responses.

Treatment	X_1	X_2	X_3	$(Y_1)^a$	$(Y_1)^b$	$(Y_2)^a$	$(Y_2)^b$
1	50	20	10	70.1	71.1	68.6	70.0
2	50	40	5	72.7	71.6	63.9	66.4
3	50	40	15	73.1	71.4	66.8	68.8
4	50	60	10	71.4	71.4	63.1	69.9
5	60	20	5	70.8	70.2	65.5	69.2
6	60	20	15	71.1	72.1	67.8	70.9
7	60	40	10	72.3	71.4	66.8	76.1
8	60	60	5	72.2	69.7	67.5	68.1
9	60	60	15	71.9	69.4	72.5	66.8
10	70	20	10	67.7	68.2	73.2	71.0
11	70	40	5	67.1	70.6	76.4	73.2
12	70	40	15	66.6	68.7	72.9	74.0
13	70	60	10	68.6	71.5	72.8	71.5
14	60	40	10	72.3	71.4	66.8	76.1
15	60	40	10	72.3	71.4	66.8	76.1

X_I – Concentration of aqueous alcohol, %
X_2 – Temperature of extraction, °C
X_3 – Time of extraction, minutes

Y_1, Y_2 – Protein content and yield of the concentrates produced by aqueous-alcohol washing in % d.b.; [a]Isopropanol; [b]Ethanol

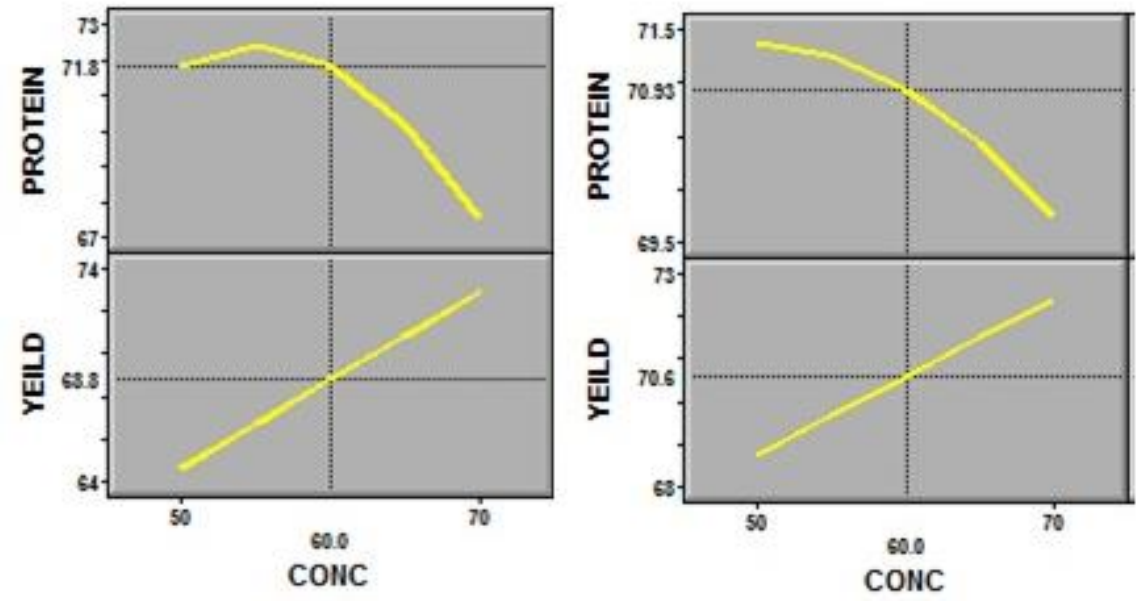

Figure 4.1: Relationships between alcohol concentration and the yield and protein contents of protein concentrates prepared from air-classified pea protein by washing with aqueous-isopropanol and aqueous-ethanol, respectively.

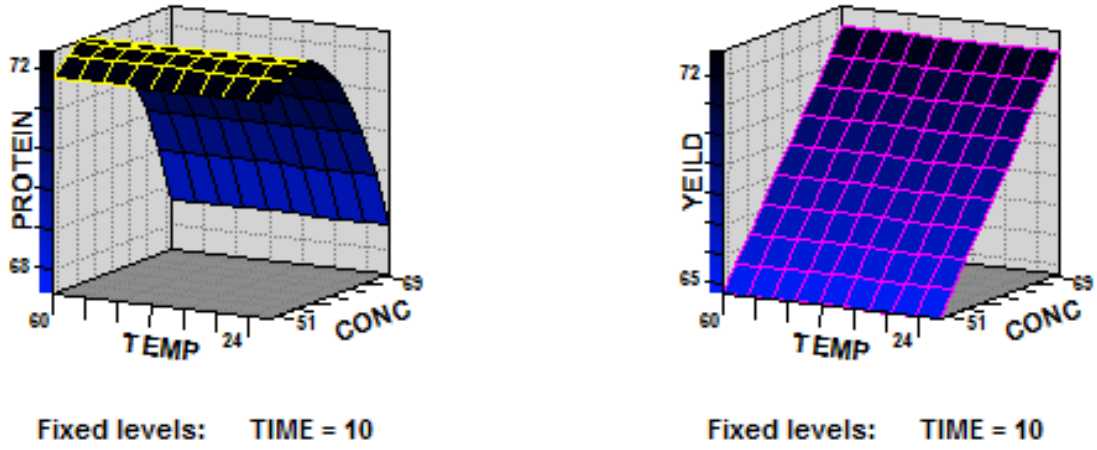

Figure 4.2a: The effects of alcohol concentration and extraction temperature on the protein content and yield of protein concentrates prepared from air-classified pea protein by washing with aqueous-isopropanol.

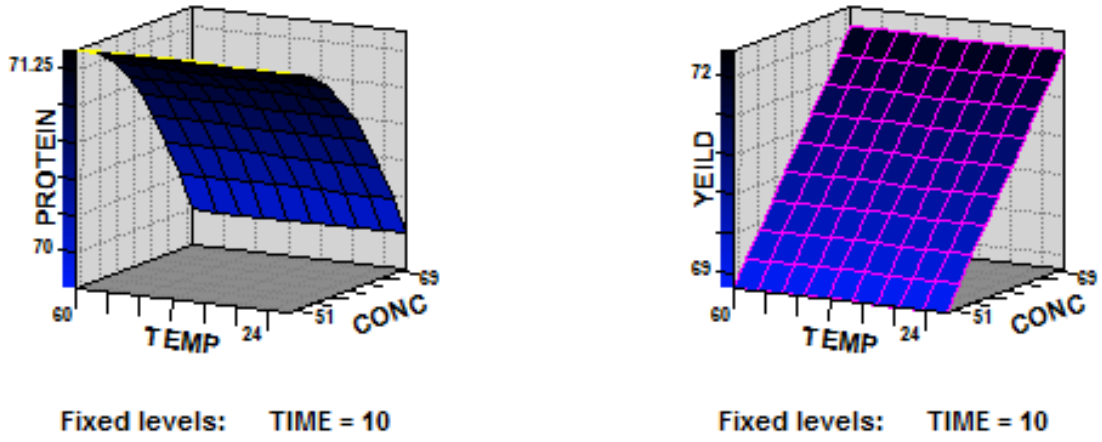

Figure 4.2b: The effects of alcohol concentration and temperature on the protein content and yield of protein concentrates prepared from air-classified pea protein by washing with aqueous-ethanol.

The statistical response Y_1 (protein content) for concentrates prepared using aqueous-isopropanol as solvent produced an R-squared value of 0.9089 and a P-value of 0.03, whereas Y_1 for aqueous-ethanol produced an R-squared value of 0.6047 and a P-value of 0.60. This indicates that 90% of the variability can be explained by the model in the case of aqueous-isopropanol as the extraction solvent, whereas the model could only explain 60% of the variability in the case of aqueous-ethanol-washed products. Hence, the model was not adequate to describe the behaviour of the protein concentrates produced by aqueous-ethanol washing. However, the model could be used to study response tendencies.

The statistical response Y_2 (yield) for concentrates prepared using aqueous-isopropanol as extraction solvent yielded an R-squared value of 0.8572 and a P-value of 0.09, whereas Y_2 for aqueous-ethanol-washed products produced an R-squared value of 0.8909 and a P-value of 0.05.

4.1.1.4 Canonical Analysis

In order to examine the shape of curvature and determine if the stationary point was a maximal, minimal or saddle point, canonical analysis was performed. The aim was to find combinations that maximized the protein content and yield of concentrates produced by aqueous-alcohol washing. The combinations can only be taken into consideration if the set of treatments lies within the region of the experimental design and the stationary point is a maximum or a minimum. A saddle point is an indication that another method must be employed to find

combinations to optimize the design. Canonical analysis of protein content values for aqueous-isopropanol-washed concentrates produced negative Eigen values, indicating that the stationary point was a maximum and that a treatment combination of 55% aqueous-isopropanol, 50°C and 11 min would produce a concentrate containing 73% protein. In the case of aqueous-ethanol-washed concentrates, the stationary point was a saddle point and hence the estimated surface did not have a distinguishable optimum.

The inverse was found in the case of the yield (Y_2) of concentrates prepared using aqueous-isopropanol and aqueous-ethanol. The stationary point for aqueous-isopropanol-washed concentrates was a saddle point and the treatment combination for highest yield was outside the region of the experimental design. Canonical analysis for yield values of aqueous-ethanol- washed concentrates produced a maximal stationary point with an ideal combination of 65% ethanol, 40°C and 11 minutes.

4.1.1.5 Ridge Analysis

Canonical analysis of protein content values for aqueous-ethanol-washed concentrates and yield values for aqueous-isopropanol-washed concentrates yielded saddle stationary points during process optimization of aqueous-alcohol washing. Thus, to seek the estimated optimum processing conditions inside a spherical region of experimentation, ridge analysis was conducted. The ridge analysis of the maximum response for Y_1 (protein content) for aqueous-isopropanol-washed protein concentrates indicated that the highest protein content could be achieved using a treatment combination of 55% aqueous-isopropanol, 50°C and 11 minutes, which was in agreement with the values from canonical analysis. Aqueous-ethanol-washed concentrates with the highest protein content could be obtained using a combination of 52% aqueous-ethanol, 32°C and 12 minutes. Ridge analysis for yield (Y_2) values for protein concentrates produced by aqueous-isopropanol washing indicated that the highest yield could be achieved using a combination of 70% aqueous-isopropanol, 44°C and 10 minutes. For aqueous-ethanol-washed concentrates, the optimum combination was found to be 65% aqueous-ethanol, 40°C and 11 minutes.

4.1.2 Compositional Analysis

The concentrations of protein, starch, crude fat, total lipid and ash in protein concentrates prepared by aqueous-alcohol (ethanol or isopropanol) washing of air-classified pea protein are presented in Table 4.3. All concentrates were true protein concentrates (protein concentration >65% on a dry weight basis) and exhibited substantially higher protein contents than the starting material. Increasing concentrations of alcohol negatively affected the protein contents of the concentrates. In other words, concentrates prepared using a higher concentration of alcohol in the extraction solvent produced a protein concentrate of lower protein content. Concentrates prepared from either alcohol (ethanol or isopropanol) were similar in protein content. Protein contents of aqueous-ethanol-washed concentrates ranged from 68.2-72.1% (d.b.), whereas those of aqueous-isopropanol-washed concentrates ranged from 66.6-73.1% (d.b.). Protein recoveries in the concentrates, expressed as a percentage of the protein contents of air-classified pea protein, ranged from 83.6-97.8% and 81.3-93.9% for aqueous-ethanol-washed and aqueous-isopropanol-washed concentrates, respectively.

Aqueous-ethanol-washed and aqueous-isopropanol-washed concentrates exhibited higher starch contents than the starting material, due to the removal of soluble materials. Aqueous-isopropanol-washed concentrates were higher in starch than aqueous-ethanol-washed concentrates for all corresponding treatment combinations, and had almost twice the starch content of the staring material. The starch contents of aqueous-ethanol-washed and aqueous-isopropanol-washed samples ranged from 3.9-6.2% and 5.8-7.6%, respectively.

The concentrations of crude fat and total lipid in protein concentrates were lower than those of the starting material, air-classified pea protein, more so in the case of aqueous-isopropanol-washed concentrates. Crude fat and total lipid contents of aqueous-ethanol-washed samples ranged from 1.2-2.4% (d.b.) and 3.8-5.2% (d.b.), respectively, whereas aqueous-isopropanol was found to be more effective than aqueous-ethanol for all corresponding treatment combinations in the removal of crude fat and total lipid. Aqueous-isopropanol-washed concentrates contained 0.3-0.8% (d.b.) and 1.3-3.0% (d.b.) of crude fat and total lipid, respectively.

The ash contents of aqueous-ethanol-washed and aqueous-isopropanol-washed samples for corresponding treatment combinations were very similar and ranged from 4.4-5.5% (d.b.) and 4.3-5.9% (d.b.), respectively. It was observed that concentrates washed at higher concentrations of

alcohol were higher in ash; this trend was observed for both aqueous-ethanol-washed and aqueous-isopropanol-washed products.

Table 4.3. Composition (%, dry basis) of protein concentrates prepared from air-classified pea protein (ACPP) by aqueous-alcohol (ethanol or isopropanol) washing.

Treatment	Protein (%)	Starch (%)	Crude fat (%)	Total lipid (%)	Ash (%)
ACPP[1]	55.5 ± 0.1[3]	3.6 ± 0.1	2.1 ± 0.0	7.5 ± 0.1	6.3 ± 0.1
EtOH-washed					
50-20-10[2]	71.1 ± 0.2	5.3 ± 0.2	1.9 ± 0.0	4.6 ± 0.5	4.5 ± 0.0
50-40-5	71.6 ± 0.2	5.8 ± 0.0	1.1 ± 0.0	4.0 ± 0.6	4.6 ± 0.0
50-40-15	71.4 ± 0.1	6.0 ± 0.2	1.6 ± 0.4	5.1 ± 0.2	4.4 ± 0.1
50-60-10	71.4 ± 0.3	6.2 ± 0.2	1.6 ± 0.0	4.7 ± 0.3	4.5 ± 0.1
60-20-5	70.2 ± 0.4	5.7 ± 0.3	2.0 ± 0.0	4.8 ± 0.5	4.8 ± 0.0
60-20-15	72.1 ± 0.1	4.5 ± 0.2	2.0 ± 0.0	5.2 ± 0.5	4.9 ± 0.0
60-40-10	71.4 ± 0.4	5.1 ± 0.0	1.8 ± 0.0	5.2 ± 0.3	5.0 ± 0.0
60-60-5	69.7 ± 0.9	5.2 ± 0.2	2.0 ± 0.0	4.1 ± 0.0	4.8 ± 0.1
60-60-15	69.4 ± 0.3	4.5 ± 0.1	2.0 ± 0.0	4.4 ± 0.0	4.7 ± 0.1
70-20-10	68.2 ± 0.2	3.9 ± 0.2	1.7 ± 0.0	4.7 ± 0.7	5.4 ± 0.2
70-40-5	70.6 ± 0.6	4.1 ± 0.2	1.8 ± 0.0	4.8 ± 0.7	5.5 ± 0.1
70-40-15	68.7 ± 0.6	4.9 ± 0.2	1.2 ± 0.1	4.7 ± 0.1	5.1 ± 0.8
70-60-10	71.5 ± 0.3	4.4 ± 0.2	1.9 ± 0.1	3.8 ± 0.3	5.5 ± 0.1
IPA-washed					
50-20-10[5]	70.1 ± 0.3	6.7 ± 0.0	0.8 ± 0.0	2.3 ± 0.3	4.3 ± 0.5
50-40-5	72.7 ± 0.4	7.2 ± 0.1	0.4 ± 0.0	2.8 ± 0.0	4.4 ± 0.1
50-40-15	73.1 ± 0.3	6.8 ± 0.0	0.5 ± 0.1	3.0 ± 0.2	4.5 ± 0.0
50-60-10	71.4 ± 0.1	6.9 ± 0.1	0.5 ± 0.1	2.6 ± 0.2	4.4 ± 0.1
60-20-5	70.8 ± 0.4	7.0 ± 0.1	0.7 ± 0.0	2.3 ± 0.2	4.7 ± 0.6
60-20-15	71.1 ± 0.3	7.0 ± 0.0	0.7 ± 0.0	2.7 ± 0.1	5.1 ± 0.0
60-40-10	72.3 ± 0.4	7.6 ± 0.2	0.7 ± 0.0	2.9 ± 0.3	5.1 ± 0.1
60-60-5	72.2 ± 0.3	6.5 ± 0.2	0.7 ± 0.0	2.7 ± 0.1	5.0 ± 0.1
60-60-15	71.9 ± 0.7	6.7 ± 0.2	0.5 ± 0.1	2.2 ± 0.1	4.9 ± 0.4
70-20-10	67.7 ± 0.3	6.3 ± 0.1	0.4 ± 0.0	1.9 ± 0.2	5.8 ± 0.0
70-40-5	67.1 ± 0.4	5.8 ± 0.0	0.8 ± 0.0	1.8 ± 0.0	5.9 ± 0.1
70-40-15	66.6 ± 0.2	6.2 ± 0.1	0.7 ± 0.0	1.3 ± 0.3	5.8 ± 0.0
70-60-10	68.6 ± 0.5	6.1 ± 0.3	0.3 ± 0.0	1.8 ± 0.1	5.1 ± 0.1

[1] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.

[2] Values are averages of two replicates ± standard deviation.

4.1.3 Functional Analysis

The functional characteristics of protein concentrates prepared by aqueous-ethanol-washing or aqueous-isopropanol washing of air-classified pea protein are presented in Tables 4.4, 4.5, 4.6 and 4.7.

Water holding capacity (WHC) and oil holding capacity (OHC) were similar for all concentrates. Neither the alcohol used, i.e. ethanol or isopropanol, nor the concentration of aqueous-alcohol, had any effect on these functional properties. All concentrates exhibited higher WHCs in comparison to the starting material, air-classified pea protein. A commercial soy protein concentrate, Arcon S, had the highest WHC at 4.2 mL/g, whereas Arcon F had lower WHC than all concentrates prepared in this study from air-classified pea protein. The OHC values of the commercial samples were slightly lower than those of air-classified pea protein and all of the concentrates prepared in this study.

As for WHC and OHC, emulsion activity (EA) values were similar for all concentrates prepared in this study, and protein concentrates exhibited improved EAs compared to the starting material, and were similar in EA to commercial concentrates. The emulsion stability (ES) of the 50-20-10 aqueous-ethanol-washed sample was similar to that of the starting material. All other concentrates exhibited ESs higher than that of the starting material.

The protein concentrates prepared by aqueous-ethanol washing exhibited similar foaming capacities (FCs) for all treatment combinations, and values were similar to that of the starting material. Aqueous-isopropanol-washed concentrates had markedly lower FCs than did the aqueous-ethanol-washed concentrates and the starting material, but again there were no marked differences in FC among the various aqueous-isopropanol-washed samples. All aqueous-alcohol-washed concentrates exhibited greater FC than did Arcon F or Arcon S. The foam stability (FS) of aqueous-isopropanol-washed samples was similar for all treatment combinations. For aqueous-ethanol-washed products, FS increased with an increase in aqueous-alcohol concentration. Nearly all alcohol-washed concentrates had somewhat higher FSs than did the starting material. The FSs of the commercial soy concentrates were similar to those of aqueous-isopropanol-washed concentrates and those of aqueous-ethanol-washed concentrates prepared at higher alcohol concentrations.

All treatments negatively affected nitrogen solubility, i.e. reduced the nitrogen solubility index (NSI). Aqueous-ethanol and aqueous-isopropanol had similar effects. Extraction

temperature also had a detrimental effect on the NSIs of the protein concentrates. The NSI of Arcon S was similar to that of the aqueous-alcohol-washed products from pea, and that of Arcon F was substantially lower in comparison to most pea protein concentrates.

4.1.4 Colour

The HunterLab values determined for air-classified pea protein and alcohol-washed protein concentrates are presented in Tables 4.8 and 4.9. With the exception of 50%-ethanol-washed concentrates, alcohol-washed concentrates were whiter (brighter) than the starting material, air-classified pea protein. Brightness tended to increase with an increase in alcohol concentration. All alcohol-washed concentrates were more green (less red) and more blue (less yellow) in colour than air-classified pea protein. Corresponding aqueous-isopropanol-washed concentrates tended to be whiter and more blue than corresponding aqueous-ethanol-washed concentrates, but similar with respect to greeness. In other words, aqueous-alcohol (ethanol or isopropanol) washing was more effective in the removal of the red and yellow colour-inducing pigments. Overall, the alcohol-washed concentrates became whiter, less red and less yellow as the concentration of aqueous-alcohol increased.

4.1.5 Oligosaccharides

Raffinose-family oligosaccharide (RFO) profiles of protein concentrates prepared from air-classified pea protein by aqueous-alcohol (ethanol or isopropanol) washing are presented in Tables 4.10 and 4.11. Stachyose was the predominant oligosaccharide in air-classified pea protein and in most alcohol-washed concentrates. The concentrates exhibited markedly lower oligosaccharide contents than did the starting material. Aqueous-isopropanol-washed concentrates were found to be somewhat more depleted in oligosaccharides than were corresponding aqueous-ethanol-washed concentrates; however, they were found to be somewhat higher in raffinose content than corresponding aqueous-ethanol-washed concentrates. It also was observed that as the concentration of alcohol in the extraction solvent increased, the ability of the solvent to extract oligosaccharides decreased, particularly with 70% aqueous-alcohol.

Table 4.4. Functionality of protein concentrates prepared from air-classified pea protein by aqueous-ethanol washing, expressed on a per gram of sample basis.

Treatment	WHC[1] (mL/g)	OHC[2] (g/g)	EA[3] (%)	ES[4] (%)	FC[5] (%)	FS[6] (%)	NSI[7] (%)
Air-classified pea protein	1.2 ± 0.1[8]	2.0 ± 0.1	29.9 ± 0.0	87.9 ± 1.0	180.0 ± 1.0	91.1 ± 0.0	77.3 ± 0.2
50-20-10[9]	3.8 ± 0.1	2.2 ± 0.1	53.7 ± 1.0	88.4 ± 1.6	181.0 ± 1.4	92.8 ± 0.7	36.5 ± 0.2
50-40-5	3.6 ± 0.0	2.1 ± 0.0	50.3 ± 0.9	95.4 ± 0.8	182.0 ± 2.8	92.3 ± 1.4	31.0 ± 0.3
50-40-15	3.7 ± 0.1	2.0 ± 0.1	56.4 ± 1.3	99.6 ± 2.0	181.0 ± 1.4	90.6 ± 0.7	31.5 ± 0.3
50-60-10	3.8 ± 0.1	2.0 ± 0.0	51.6 ± 1.2	103.3 ± 1.7	181.0 ± 1.4	92.3 ± 0.1	14.6 ± 0.3
60-20-5	3.7 ± 0.1	2.4 ± 0.1	48.7 ± 1.1	98.9 ± 0.9	170.0 ± 2.8	97.6 ± 0.0	45.0 ± 0.3
60-20-15	3.7 ± 0.0	2.5 ± 0.1	50.0 ± 1.4	104.6 ± 1.9	171.0 ± 1.4	97.1 ± 0.8	45.0 ± 0.3
60-40-10	3.9 ± 0.1	2.7 ± 0.1	51.8 ± 1.4	107.2 ± 0.8	174.0 ± 0.0	96.0 ± 0.8	32.6 ± 0.2
60-60-5	3.8 ± 0.1	2.3 ± 0.1	49.3 ± 1.1	104.1 ± 1.1	174.0 ± 2.8	95.4 ± 1.6	20.6 ± 0.3
60-60-15	3.7 ± 0.1	2.5 ± 0.1	48.6 ± 1.4	101.0 ± 1.9	173.0 ± 1.4	96.0 ± 2.4	12.2 ± 0.3
70-20-10	3.7 ± 0.1	2.7 ± 0.0	53.5 ± 1.8	98.1 ± 1.0	173.0 ± 1.4	99.4 ± 0.8	45.5 ± 0.3
70-40-5	3.8 ± 0.1	2.7 ± 0.0	52.5 ± 0.4	103.6 ± 1.7	171.0 ± 1.4	101.2 ± 0.0	38.0 ± 0.3
70-40-15	3.7 ± 0.2	2.6 ± 0.1	52.5 ± 0.0	100.5 ± 1.5	169.0 ± 1.4	102.4 ± 0.0	39.0 ± 0.3
70-60-10	3.8 ± 0.1	2.7 ± 0.2	53.0 ± 1.8	102.3 ± 0.6	171.0 ± 1.4	99.4 ± 0.8	33.1 ± 0.3
Arcon S[10]	4.2 ± 0.0	1.9 ± 0.0	53.7 ± 0.0	102.3 ± 0.1	158.0 ± 0.0	98.7 ± 0.0	36.7 ± 0.3
Arcon F[11]	2.5 ± 0.0	1.6 ± 0.0	48.7 ± 0.0	103.6 ± 0.0	130.0 ± 0.0	98.5 ± 0.0	10.3 ± 0.2

[1] WHC = Water hydration capacity.
[2] OHC = Oil holding capacity.
[3] EA = Emulsion activity.
[4] ES = Emulsion stability.
[5] FC = Foaming capacity.
[6] FS = Foam stability.
[7] NSI = Nitrogen solubility index.
[8] Mean± standard deviation, n=2.
[9] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.
[10] Arcon S = Commercial soy concentrate.
[11] Arcon F = Commercial soy concentrate.

Table 4.5. Functionality of protein concentrates prepared from air-classified pea protein by aqueous-ethanol washing, expressed on a per gram of protein basis.

Treatment	WHC[1] (mL/g)	OHC[2] (g/g)	EA[3] (%)	ES[4] (%)	FC[5] (%)	FS[6] (%)	NSI[7] (%)
Air-classified pea protein	2.2 ± 0.1[8]	3.6 ± 0.1	53.9 ± 0.0	158.4 ± 1.0	324.3 ± 1.0	164.1 ± 0.0	139.3 ± 0.2
50-20-10[9]	5.3 ± 0.1	3.1 ± 0.1	75.6 ± 1.0	124.4 ± 1.6	254.7 ± 1.4	130.6 ± 0.7	51.4 ± 0.2
50-40-5	5.0 ± 0.0	2.9 ± 0.0	70.2 ± 0.9	133.1 ± 0.8	254.0 ± 2.8	128.8 ± 1.4	43.3 ± 0.3
50-40-15	5.2 ± 0.1	2.8 ± 0.1	79.0 ± 1.3	139.6 ± 2.0	253.7 ± 1.4	127.0 ± 0.7	44.1 ± 0.3
50-60-10	5.3 ± 0.1	2.8 ± 0.0	72.3 ± 1.2	144.7 ± 1.7	253.6 ± 1.4	129.3 ± 0.1	20.5 ± 0.3
60-20-5	5.3 ± 0.1	3.4 ± 0.1	69.3 ± 1.1	140.8 ± 0.9	242.0 ± 2.8	139.0 ± 0.0	64.1 ± 0.3
60-20-15	5.1 ± 0.0	3.5 ± 0.1	69.3 ± 1.4	145.0 ± 1.9	237.1 ± 1.4	134.6 ± 0.8	62.4 ± 0.3
60-40-10	5.5 ± 0.1	3.8 ± 0.1	72.6 ± 1.4	150.2 ± 0.8	243.8 ± 0.0	134.5 ± 0.8	45.6 ± 0.2
60-60-5	5.5 ± 0.1	3.3 ± 0.1	70.7 ± 1.1	149.4 ± 1.1	249.7 ± 2.8	136.9 ± 1.6	29.6 ± 0.3
60-60-15	5.3 ± 0.1	3.6 ± 0.1	70.0 ± 1.4	145.4 ± 1.9	249.1 ± 1.4	138.2 ± 2.4	17.5 ± 0.3
70-20-10	5.4 ± 0.1	4.0 ± 0.0	78.4 ± 1.8	143.8 ± 1.0	253.6 ± 1.4	145.7 ± 0.8	66.7 ± 0.3
70-40-5	5.4 ± 0.1	3.8 ± 0.0	74.4 ± 0.4	146.8 ± 1.7	242.2 ± 1.4	143.4 ± 0.0	53.9 ± 0.3
70-40-15	5.4 ± 0.2	3.8 ± 0.1	76.4 ± 0.0	146.2 ± 1.5	245.9 ± 1.4	149.0 ± 0.0	56.7 ± 0.3
70-60-10	5.3 ± 0.1	3.8 ± 0.2	74.1 ± 1.8	143.1 ± 0.6	239.2 ± 1.4	139.0 ± 0.8	46.3 ± 0.3
Arcon S[10]	5.4 ± 0.0	2.5 ± 0.0	69.6 ± 0.0	132.5 ± 0.1	204.7 ± 0.0	127.8 ± 0.0	47.5 ± 0.3
Arcon F[11]	3.6 ± 0.0	2.3 ± 0.0	69.5 ± 0.0	147.8 ± 0.0	185.4 ± 0.0	140.5 ± 0.0	14.7 ± 0.2

[1] WHC = Water hydration capacity.
[2] OHC = Oil holding capacity.
[3] EA = Emulsion activity.
[4] ES = Emulsion stability.
[5] FC = Foaming capacity.
[6] FS = Foam stability.
[7] NSI = Nitrogen solubility index.
[8] Mean± standard deviation, n=2.
[9] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.
[10] Arcon S = Commercial soy concentrate.
[11] Arcon F = Commercial soy concentrate.

Table 4.6. Functionality of protein concentrates prepared from air-classified pea protein by aqueous-isopropanol washing, expressed on a per gram of sample basis.

Treatment	WHC[1] (mL/g)	OHC[2] (g/g)	EA[3] (%)	ES[4] (%)	FC[5] (%)	FS[6] (%)	NSI[7] (%)
Air-classified pea protein	1.2 ± 0.1[8]	2.0 ± 0.1	29.9 ± 0.0	87.9 ± 1.0	180.0 ± 1.0	91.1 ± 0.0	77.3 ± 0.2
50-20-10[9]	3.8 ± 0.1	1.9 ± 0.1	47.4 ± 0.8	104.7 ± 0.4	100.7 ± 0.9	99.3 ± 0.9	41.1 ± 0.3
50-40-5	3.8 ± 0.0	2.5 ± 0.0	49.6 ± 1.0	108.2 ± 1.0	101.4 ± 0.0	98.7 ± 0.0	33.4 ± 0.2
50-40-15	3.8 ± 0.0	2.2 ± 0.1	52.0 ± 1.4	103.8 ± 1.2	101.3 ± 0.0	98.7 ± 0.0	29.6 ± 0.1
50-60-10	3.9 ± 0.0	2.3 ± 0.1	53.2 ± 0.9	101.2 ± 1.6	100.7 ± 1.0	99.3 ± 0.9	25.3 ± 0.2
60-20-5	3.7 ± 0.1	2.8 ± 0.1	51.7 ± 0.5	103.8 ± 1.9	100.6 ± 0.9	99.4 ± 0.9	43.1 ± 0.1
60-20-15	3.7 ± 0.0	2.5 ± 0.1	50.9 ± 0.6	108.0 ± 1.4	100.6 ± 0.9	99.4 ± 0.9	47.7 ± 0.2
60-40-10	3.8 ± 0.1	2.8 ± 0.2	51.4 ± 1.3	107.2 ± 0.5	101.8 ± 0.9	98.2 ± 0.8	45.6 ± 0.2
60-60-5	3.8 ± 0.1	2.7 ± 0.4	51.2 ± 1.8	104.3 ± 1.5	101.1 ± 0.0	98.9 ± 0.0	27.4 ± 0.2
60-60-15	3.7 ± 0.0	2.7 ± 0.2	50.3 ± 1.4	102.0 ± 1.6	101.1 ± 0.0	99.5 ± 0.7	23.4 ± 0.1
70-20-10	3.7 ± 0.1	2.5 ± 0.0	49.8 ± 2.8	113.6 ± 1.6	100.5 ± 0.7	99.5 ± 0.7	57.3 ± 0.2
70-40-5	3.7 ± 0.1	2.7 ± 0.1	51.2 ± 0.9	104.5 ± 0.8	100.5 ± 0.7	98.5 ± 0.7	55.1 ± 0.4
70-40-15	3.7 ± 0.1	2.6 ± 0.1	50.9 ± 0.5	106.1 ± 1.1	101.0 ± 0.0	99.0 ± 0.0	55.8 ± 0.2
70-60-10	3.7 ± 0.1	2.4 ± 0.1	50.9 ± 1.3	99.0 ± 0.9	101.5 ± 0.7	98.5 ± 0.7	48.2 ± 0.3
Arcon S[10]	4.2 ± 0.0	1.9 ± 0.0	53.7 ± 0.0	102.3 ± 0.1	158.0 ± 0.0	98.7 ± 0.0	36.7 ± 0.3
Arcon F[11]	2.5 ± 0.0	1.6 ± 0.0	48.7 ± 0.0	103.6 ± 0.0	130.0 ± 0.0	98.5 ± 0.0	10.3 ± 0.2

[1] WHC = Water hydration capacity.
[2] OHC = Oil holding capacity.
[3] EA = Emulsion activity.
[4] ES = Emulsion stability.
[5] FC = Foaming capacity.
[6] FS = Foam stability.
[7] NSI = Nitrogen solubility index.
[8] Mean± standard deviation, n=2.
[9] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.
[10] Arcon S = Commercial soy concentrate.
[11] Arcon F = Commercial soy concentrate.

Table 4.7. Functionality of protein concentrates prepared from air-classified pea protein by aqueous-isopropanol washing, expressed on a per gram of protein basis.

Treatment	WHC[1] (mL/g)	OHC[2] (g/g)	EA[3] (%)	ES[4] (%)	FC[5] (%)	FS[6] (%)	NSI[7] (%)
Air-classified pea protein	2.2 ± 0.1[8]	3.6 ± 0.1	53.9 ± 0.0	158.4 ± 1.0	324.3 ± 1.0	164.1 ± 0.0	139.3 ± 0.2
50-20-10[9]	5.4 ± 0.1	2.7 ± 0.1	67.6 ± 1.0	149.3 ± 0.4	143.6 ± 0.9	141.6 ± 0.7	58.6 ± 0.2
50-40-5	5.2 ± 0.0	3.4 ± 0.0	68.2 ± 0.9	148.8 ± 1.0	139.5 ± 0.0	135.7 ± 1.4	45.9 ± 0.3
50-40-15	5.2 ± 0.1	3.0 ± 0.1	71.2 ± 1.3	142.1 ± 1.2	138.7 ± 0.0	135.1 ± 0.7	40.5 ± 0.3
50-60-10	5.5 ± 0.1	3.2 ± 0.0	74.5 ± 1.2	141.8 ± 1.6	141.1 ± 1.0	139.1 ± 0.1	35.4 ± 0.3
60-20-5	5.2 ± 0.1	4.0 ± 0.1	73.0 ± 1.1	146.7 ± 1.9	142.1 ± 0.9	140.4 ± 0.0	60.9 ± 0.3
60-20-15	5.2 ± 0.0	3.5 ± 0.1	71.6 ± 1.4	152.0 ± 1.4	141.6 ± 0.9	139.9 ± 0.8	67.1 ± 0.3
60-40-10	5.3 ± 0.1	3.9 ± 0.1	71.1 ± 1.4	148.3 ± 0.5	140.8 ± 0.9	135.8 ± 0.8	63.1 ± 0.2
60-60-5	5.3 ± 0.1	3.7 ± 0.1	70.9 ± 1.1	144.4 ± 1.5	140.0 ± 0.0	137.0 ± 1.6	37.9 ± 0.3
60-60-15	5.1 ± 0.1	3.8 ± 0.1	70.0 ± 1.4	141.9 ± 1.6	140.7 ± 0.0	138.4 ± 2.4	32.6 ± 0.3
70-20-10	5.5 ± 0.1	3.7 ± 0.0	73.6 ± 1.8	167.8 ± 1.6	148.4 ± 0.7	147.0 ± 0.8	84.6 ± 0.3
70-40-5	5.5 ± 0.1	4.0 ± 0.0	76.4 ± 0.4	155.9 ± 0.8	149.9 ± 0.7	146.9 ± 0.0	82.2 ± 0.3
70-40-15	5.6 ± 0.2	3.9 ± 0.1	76.4 ± 0.0	159.3 ± 1.1	151.7 ± 0.0	148.7 ± 0.0	83.8 ± 0.3
70-60-10	5.4 ± 0.1	3.5 ± 0.2	74.2 ± 1.8	144.3 ± 0.9	148.0 ± 0.7	143.6 ± 0.8	70.3 ± 0.3
Arcon S[10]	5.4 ± 0.0	2.5 ± 0.0	69.6 ± 0.0	132.5 ± 0.1	204.7 ± 0.0	127.8 ± 0.0	47.5 ± 0.3
Arcon F[11]	3.6 ± 0.0	2.3 ± 0.0	69.5 ± 0.0	147.8 ± 0.0	185.4 ± 0.0	140.5 ± 0.0	14.7 ± 0.2

[1] WHC = Water hydration capacity.
[2] OHC = Oil holding capacity.
[3] EA = Emulsion activity.
[4] ES = Emulsion stability.
[5] FC = Foaming capacity.
[6] FS = Foam stability.
[7] NSI = Nitrogen solubility index.
[8] Mean± standard deviation, n=2.
[9] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.
[10] Arcon S = Commercial soy concentrate.
[11] Arcon F = Commercial soy concentrate.

Table 4.8. HunterLab colour values for protein concentrates prepared from air classified pea protein by aqueous-ethanol washing.

Treatments	L[1]	a[2]	b[3]
Air-classified pea protein	86.7 ± 0.1[4]	6.3 ± 0.1	19.9 ± 0.1
50-20-10[5]	85.6 ± 0.1	0.8 ± 0.0	13.8 ± 0.1
50-40-5	82.9 ± 0.2	1.3 ± 0.1	16.4 ± 0.1
50-40-15	82.1 ± 0.1	1.4 ± 0.0	16.0 ± 0.1
50-60-10	83.0 ± 0.1	1.0 ± 0.0	15.7 ± 0.1
60-20-5	87.4 ± 0.3	0.6 ± 0.1	12.4 ± 0.3
60-20-15	88.6 ± 0.1	0.5 ± 0.0	10.3 ± 0.0
60-40-10	88.1 ± 0.2	0.4 ± 0.0	11.5 ± 0.1
60-60-5	86.7 ± 0.2	0.4 ± 0.0	13.4 ± 0.0
60-60-15	86.0 ± 0.4	0.2 ± 0.1	12.4 ± 0.5
70-20-10	88.5 ± 0.2	0.6 ± 0.0	9.8 ± 0.1
70-40-5	88.1 ± 0.0	0.4 ± 0.0	9.8 ± 0.0
70-40-15	88.6 ± 0.8	0.3 ± 0.1	10.1 ± 0.2
70-60-10	89.4 ± 0.2	-0.1± 0.0	9.9 ± 0.1

[1] L = 100 white, 0 black.
[2] a = + red, - green.
[3] b = + yellow, - blue.
[4] Mean± standard deviation, n=2.
[5] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.

Table 4.9. HunterLab colour values for protein concentrates prepared from air classified pea protein by aqueous-isopropanol washing.

Treatments	L[1]	a[2]	b[3]
Air-classified pea protein	86.7 ± 0.1[4]	6.3 ± 0.1	19.9 ± 0.1
50-20-10[5]	89.6 ± 0.1	0.4 ± 0.2	9.4 ± 0.5
50-40-5	88.8 ± 0.1	0.5 ± 0.0	9.4 ± 0.1
50-40-15	88.2 ± 0.1	0.4 ± 0.0	10.2 ± 0.2
50-60-10	87.2 ± 0.7	0.2 ± 0.1	11.1 ± 0.9
60-20-5	89.5 ± 0.0	0.9 ± 0.0	9.0 ± 0.0
60-20-15	89.9 ± 0.2	0.1 ± 0.0	8.4 ± 0.3
60-40-10	89.8 ± 0.6	0.4 ± 0.1	8.6 ± 0.5
60-60-5	89.3 ± 0.0	0.1 ± 0.0	8.9 ± 0.1
60-60-15	89.1 ± 0.4	0.1 ± 0.0	9.1 ± 0.3
70-20-10	90.4 ± 0.1	0.7 ± 0.1	8.3 ± 0.2
70-40-5	90.4 ± 0.1	0.3 ± 0.1	8.0 ± 0.0
70-40-15	90.5 ± 0.0	0.3 ± 0.0	8.0 ± 0.0
70-60-10	90.3 ± 0.1	0.0 ± 0.1	8.2 ± 0.1

[1] L = 100 white, 0 black.
[2] a = + red, - green.
[3] b = + yellow, - blue.
[4] Mean± standard deviation, n=2.
[5] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.

Table 4.10. Oligosaccharide profiles (%, dry basis) of protein concentrates prepared from air-classified pea protein by aqueous-ethanol washing.

Treatments	Raffinose (%)	Stachyose (%)	Verbascose (%)	Total RFO[1]
Air-classified pea protein	2.3 ± 0.0[2]	4.4 ± 0.0	1.7 ± 0.0	8.4 ± 0.2
50-20-10[5]	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
50-40-5	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.0	0.7 ± 0.2
50-40-15	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.6 ± 0.1
50-60-10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
60-20-5	0.2 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.8 ± 0.2
60-20-15	0.3 ± 0.0	0.8 ± 0.1	0.2 ± 0.0	1.3 ± 0.3
60-40-10	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
60-60-5	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.5 ± 0.1
60-60-15	0.2 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.8 ± 0.1
70-20-10	1.0 ± 0.1	2.9 ± 0.2	1.3 ± 0.2	5.2 ± 0.2
70-40-5	0.7 ± 0.1	1.9 ± 0.1	0.5 ± 0.1	3.1 ± 0.1
70-40-15	0.6 ± 0.0	1.6 ± 0.1	0.4 ± 0.2	2.6 ± 0.1
70-60-10	0.5 ± 0.0	1.2 ± 0.0	0.3 ± 0.0	2.0 ± 0.1

[1] RFO = Raffinose family oligosaccharides.
[2] Mean± standard deviation, n=2.
[3] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.

Table 4.11. Oligosaccharide profiles (%, dry basis) of protein concentrates prepared from air-classified pea protein by aqueous-ethanol washing.

Treatments	Raffinose (%)	Stachyose (%)	Verbascose (%)	Total RFO[1]
Air-classified pea protein	2.3 ± 0.0[2]	4.4 ± 0.0	1.7 ± 0.0	8.4 ± 0.2
50-20-10[5]	0.1 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.5 ± 0.1
50-40-5	0.2 ± 0.0	0.4 ± 0.2	0.1 ± 0.0	0.7 ± 0.1
50-40-15	0.2 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.8 ± 0.1
50-60-10	0.2 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	0.6 ± 0.1
60-20-5	0.6 ± 0.0	1.8 ± 0.0	1.0 ± 0.1	3.4 ± 0.1
60-20-15	0.4 ± 0.0	1.4 ± 0.1	0.4 ± 0.0	2.2 ± 0.1
60-40-10	0.4 ± 0.0	1.5 ± 0.0	0.6 ± 0.0	2.5 ± 0.1
60-60-5	0.4 ± 0.1	1.4 ± 0.0	0.7 ± 0.0	2.5 ± 0.1
60-60-15	0.4 ± 0.0	1.5 ± 0.1	0.6 ± 0.0	2.5 ± 0.1
70-20-10	1.3 ± 0.1	4.1 ± 0.2	2.8 ± 0.6	8.2 ± 0.1
70-40-5	1.4 ± 0.1	4.1 ± 0.1	2.2 ± 0.1	7.7 ± 0.1
70-40-15	1.0 ± 0.1	3.5 ± 0.1	1.9 ± 0.0	6.4 ± 0.1
70-60-10	0.9 ± 0.2	3.3 ± 0.0	1.6 ± 0.2	5.8 ± 0.1

[1] RFO = Raffinose family oligosaccharides.
[2] Mean± standard deviation, n=2.
[3] 50-20-10 = 50% aqueous-alcohol, 20°C, 10-minute extraction time.

4.2 Comparative study of the yield, composition and functionality of protein concentrates prepared from air-classified pea, lentil, fababean and navy bean protein fractions by aqueous-ethanol washing

4.2.1 Concentrate yield and composition

The composition and yield of protein products prepared from air-classified protein fractions of pea, lentil, fababean and navy bean are presented in Table 4.12. The yields of all products were similar for both extraction treatments. The optimal extraction conditions identified for the yield of aqueous-ethanol-washed pea protein concentrates (65% aqueous ethanol, 40°C, 11 minutes) produced higher product yields from lentil, fababean and navy bean than did conditions identified as optimal for the protein content of pea protein concentrates (52% aqueous ethanol, 32°C, 12 minutes).

With the exception of products from lentil and navy bean, all of the protein products contained over 65% protein (dry weight basis) and could be classified as true protein concentrates. The protein contents of the air-classified protein fractions increased by a factor of 1.2-1.3 upon aqueous-ethanol washing. The levels of the proximate components in the protein products tended to reflect their levels in the corresponding air-classified fractions. Extraction conditions identified as optimal for protein content in pea protein concentrates yielded products higher in protein for pea and fababean, and lower in protein for lentil and navy bean. The pea protein concentrates had the highest protein contents. The relatively low protein content in the navy bean concentrates reflected the low protein content of the air-classified fraction from navy bean (41.5% d.b). Protein recoveries in the concentrates expressed as a percentage of the protein contents of the respective air-classified protein fractions were 89.9% and 90.6% for pea, 81.2% and 90.9% for lentil, 81.8% and 87.1% for fababean, and 87.1% and 90.7% for navy bean. In all cases, protein recoveries were higher for concentrates prepared under conditions determined to be optimal for the yield of pea protein concentrates.

The starch contents of all aqueous-ethanol-washed concentrates were higher than those of the starting materials. Lentil products exhibited the highest starch contents among all concentrates, and pea the lowest. The fababean concentrate prepared using 65%-aqueous-ethanol, 40°C and 11 minutes exhibited the highest increase in starch content upon aqueous-ethanol washing.

All concentrates were at least somewhat depleted in their crude fat, total lipid and ash contents upon aqueous-ethanol washing. The aqueous-ethanol-washed products from navy bean exhibited the lowest levels of both crude fat and total lipid. Protein products from pea and lentil were similar in crude fat, but higher in this respect than products from fababean and navy bean, and the product from fababean was higher in crude fat than the product from navy bean. Similar trends were observed for total lipid in aqueous-ethanol-washed protein products, with the exception that products from lentil and fababean had similar total lipid contents and were lower in total lipid than products from pea, and higher in total lipid than products from navy bean. With respect to the ash content of products prepared under conditions identified as optimal for the protein content of pea protein concentrates, the concentrate from pea exhibited the highest level of ash and the navy bean product, the lowest. Ash contents were higher in products prepared under conditions identified as optimal for the yield of pea protein concentrates, with pea, fababean and navy bean exhibiting similar levels, and higher levels than in the lentil protein product.

4.2.2 Functional Analysis

The functionality of protein concentrates prepared by aqueous-ethanol washing of air-classified pea, lentil, fababean and navy bean protein fractions is described in Tables 4.13 and 4.14.

Products prepared from air-classified pea protein exhibited higher WHCs than did those from lentil, fababean or navy bean when prepared using conditions identified as optimal for the protein content or yield of pea protein concentrates. The WHCs of the products from lentil, fababean and navy bean were similar. The WHCs of pea protein concentrates were similar to that of Arcon S, and values for the lentil, fababean and navy bean products were similar to that of Arcon F. All protein products, including both of the commercial soy concentrates, exhibited similar OHCs.

The concentrates from pea exhibited the highest EA values, similar to those of the commercial soy concentrates. The EA values for the lentil, fababean and navy bean products were similar. Pea protein concentrates exhibited the highest ES values, similar to those of the soy products. Once again, the products from lentil, fababean and navy bean had similar values.

The FCs of all of the aqueous-ethanol-washed products were higher than those of the commercial soy concentrates. The FC of the pea protein concentrates was the same for both extraction treatments. The extraction conditions identified as optimal for the yield of pea protein

concentrates yielded protein products from lentil, fababean and navy bean having higher FC compared to corresponding products prepared using extraction conditions identified as optimal for protein content in pea protein concentrates, particularly in the case of lentil. With the exception of the lentil product prepared under extraction conditions identified as optimal for protein content in pea protein concentrates, products from lentil, fababean and navy bean had higher FCs than did the pea protein concentrates. The FSs of the aqueous-ethanol-washed products and the commercial soy concentrates were similar, with the exception of the products from lentil and navy bean prepared under conditions identified as optimal for protein content in pea protein concentrates.

As observed in the previous study, all aqueous-ethanol-washed products exhibited much lower NSIs than did the corresponding starting materials. The NSI of one of the commercial soy concentrates was higher than the values for all of the aqueous-ethanol-washed products, and the NSI for the other soy concentrate was lower than those of the aqueous-ethanol-washed products.

4.2.3 Colour

The HunterLab values determined for protein concentrates prepared by aqueous-ethanol washing of air-classified pea, lentil, fababean and navy bean fractions are presented in Table 4.15. Overall, the aqueous-ethanol-washed products were whiter, less red and less yellow than the respective starting materials. Loss of colour was more evident in samples prepared using extraction conditions identified as optimal for the yield of pea protein concentrates. In other words, a higher concentration of alcohol in the extracting solvent enhanced the removal of coloured compounds from the air-classified protein fractions.

4.2.4 Oligosaccharides

The concentrations of raffinose, stachyose and verbascose determined in protein concentrates prepared by aqueous-ethanol washing of air-classified pea, lentil, fababean and navy bean fractions are presented in Table 4.16. Stachyose was the most abundant oligosaccharide in all aqueous-ethanol-washed fractions and all starting materials. It was observed that the extraction conditions identified as optimal for the protein content of pea protein concentrates were more effective in extracting oligosaccharides from air-classified protein fractions than were the conditions identified as optimal for yield.

Table 4.12. Yield and composition of aqueous-ethanol-washed protein products prepared from air-classified pea, lentil, fababean and navy bean protein fractions using conditions identified as optimal for protein content and yield of pea protein concentrates.

Treatment	Product	Protein (%)	Starch (%)	Crude Fat (%)	Total Lipid (%)	Ash (%)	Yield (%)
Air-classified protein	Pea	55.5 ± 0.2[1]	3.6 ± 0.1	2.1 ± 0.5	7.5 ± 0.1	6.3 ± 0.1	-
	Lentil	49.1 ± 0.4	15.2 ± 0.1	1.6 ± 0.4	5.5 ± 0.4	4.5 ± 0.0	-
	Fababean	54.2 ± 0.1	6.6 ± 0.1	1.4 ± 0.1	6.7 ± 0.3	7.1 ± 0.0	-
	Navy bean	41.5 ± 0.3	8.3 ± 0.1	1.5 ± 0.1	6.2 ± 0.1	8.0 ± 0.0	-
52-32-12[2]	Pea	73.7 ± 0.4	4.8 ± 0.1	1.7 ± 0.4	4.7 ± 0.3	4.7 ± 0.4	67.7 ± 0.4
	Lentil	61.3 ± 0.6	16.9 ± 0.8	1.5 ± 0.1	2.4 ± 0.3	2.6 ± 0.1	65.0 ± 0.2
	Fababean	66.2 ± 0.1	8.9 ± 0.1	0.9 ± 0.0	2.0 ± 0.0	3.6 ± 0.5	67.0 ± 0.4
	Navy bean	52.3 ± 0.5	10.4 ± 0.1	0.4 ± 0.0	1.4 ± 0.1	3.7 ± 0.4	66.7 ± 0.1
65-40-11[3]	Pea	69.2 ± 0.2	4.7 ± 0.2	1.8 ± 0.1	5.0 ± 0.4	4.9 ± 0.2	72.7 ± 0.2
	Lentil	64.7 ± 0.2	15.9 ± 0.1	1.5 ± 0.1	2.8 ± 0.0	3.4 ± 0.1	69.0 ± 0.3
	Fababean	66.9 ± 0.4	10.4 ± 0.2	1.0 ± 0.0	2.1 ± 0.1	5.0 ± 0.0	70.6 ± 0.0
	Navy bean	53.6 ± 0.3	12.2 ± 0.0	0.3 ± 0.0	1.4 ± 0.1	5.6 ± 0.2	70.2 ± 0.1

[1] Mean± standard deviation, n=2.
[2] 52-32-12 = 52% aqueous-alcohol, 32°C, 12 minute extraction time (conditions optimal for protein content)
[3] 65-40-11 = 65% aqueous-alcohol, 40°C, 11 minute extraction time (conditions optimal for yield)

Table 4.13. Functionality of aqueous-ethanol-washed protein products prepared from air-classified pea, lentil, fababean and navy bean protein fractions using conditions identified as optimal for protein content and yield of pea protein concentrates, and expressed on a per gram of sample basis.

Treatment	Product	WHC[1] (mL/g)	OHC[2] (g/g)	EA[3] (%)	ES[4] (%)	FC[5] (%)	FS[6] (%)	NSI[7] (%)
Air-classified protein	Pea	1.2 ± 0.1[8]	2.0 ± 0.0	29.9 ± 0.0	87.9 ± 1.0	180.0 ± 0.0	91.1 ± 0.0	77.3 ± 0.2
	Lentil	1.0 ± 0.0	2.1 ± 0.0	25.8 ± 0.4	92.0 ± 0.9	194.0 ± 0.1	97.9 ± 0.0	83.7 ± 0.1
	Fababean	1.2 ± 0.0	2.3 ± 0.1	32.0 ± 0.6	96.9 ± 0.7	216.0 ± 0.0	98.1 ± 0.1	79.1 ± 0.2
	Navy bean	1.0 ± 0.1	1.9 ± 0.1	33.0 ± 0.2	87.5 ± 0.3	182.0 ± 0.0	98.9 ± 0.1	60.9 ± 0.1
52-32-12[9]	Pea	3.7 ± 0.1	2.1 ± 0.1	55.7 ± 1.3	99.6 ± 2.1	174.0 ± 0.1	97.7 ± 0.1	31.7 ± 0.1
	Lentil	1.9 ± 0.1	2.0 ± 0.1	24.6 ± 0.9	88.5 ± 0.6	152.0 ± 0.0	85.5 ± 0.0	13.3 ± 0.1
	Fababean	2.2 ± 0.0	2.4 ± 0.2	24.8 ± 1.9	85.6 ± 0.4	170.0 ± 0.0	94.1 ± 0.0	33.0 ± 0.2
	Navy bean	2.3 ± 0.1	2.3 ± 0.1	24.6 ± 0.8	91.8 ± 0.7	186.0 ± 0.4	89.2 ± 0.1	22.1 ± 0.1
65-40-11[10]	Pea	3.9 ± 0.1	2.7 ± 0.1	52.0 ± 1.2	106.7 ± 0.4	174.0 ± 0.1	95.4 ± 0.1	30.8 ± 0.2
	Lentil	1.9 ± 0.2	2.2 ± 0.1	24.6 ± 0.6	90.2 ± 0.6	192.0 ± 0.0	96.3 ± 0.7	14.0 ± 0.1
	Fababean	2.2 ± 0.1	2.4 ± 0.1	24.9 ± 0.2	90.1 ± 0.1	190.0 ± 0.4	95.8 ± 1.4	15.2 ± 0.3
	Navy bean	2.2 ± 0.1	2.4 ± 0.1	24.5 ± 0.0	86.8 ± 0.3	200.0 ± 0.0	98.0 ± 0.0	18.0 ± 0.2
Commercial concentrates	Arcon S[11]	4.2 ± 0.0	1.9 ± 0.0	53.7 ± 0.0	102.3 ± 0.1	158.0 ± 0.0	98.7 ± 0.0	36.7 ± 0.3
	Arcon F[12]	2.5 ± 0.0	1.6 ± 0.0	48.7 ± 0.0	103.6 ± 0.0	130.0 ± 0.0	98.5 ± 0.0	10.3 ± 0.2

[1] WHC = Water hydration capacity.
[2] OHC = Oil holding capacity.
[3] EA = Emulsion activity.
[4] ES = Emulsion stability.
[5] FC = Foaming capacity.
[6] FS = Foam stability.
[7] NSI = Nitrogen solubility index.
[8] Mean± standard deviation, n=2.
[9] 52-32-12 = 52% aqueous-alcohol, 32°C, 12-minute extraction time; optimal protein
[10] 65-40-11 = 65% aqueous-alcohol, 40°C, 11-minute extraction time; optimal yield
[11] Arcon S = Commercial soy concentrate.
[12] Arcon F = Commercial soy concentrate

Table 4.14. Functionality of aqueous-ethanol-washed protein products prepared from air-classified pea, lentil, fababean and navy bean protein fractions using conditions identified as optimal for protein content and yield of pea protein concentrates, and expressed on a per gram of protein basis.

Treatment	Product	WHC[1] (mL/g)	OHC[2] (g/g)	EA[3] (%)	ES[4] (%)	FC[5] (%)	FS[6] (%)	NSI[7] (%)
Air-classified protein	Pea	2.2 ± 0.1[8]	3.6 ± 0.0	53.9 ± 0.0	158.4 ± 1.0	324.3 ± 0.0	164.1 ± 0.0	139.3± 0.2
	Lentil	2.0 ± 0.0	4.3 ± 0.0	52.5 ± 0.4	187.4 ± 0.9	395.1 ± 0.1	199.4 ± 0.0	170.5± 0.1
	Fababean	2.2 ± 0.0	4.2 ± 0.1	59.0 ± 0.6	178.8 ± 0.7	398.5 ± 0.0	181.0 ± 0.1	145.9± 0.2
	Navy bean	2.4 ± 0.1	4.6 ± 0.1	79.5 ± 0.2	210.8 ± 0.3	438.6 ± 0.0	238.3 ± 0.1	146.7± 0.1
52-32-12[9]	Pea	5.0 ± 0.1	2.8 ± 0.1	75.6 ± 1.3	135.1 ± 2.1	236.1 ± 0.1	132.6 ± 0.1	43.0 ± 0.1
	Lentil	3.1 ± 0.1	3.3 ± 0.1	40.1 ± 0.9	144.4 ± 0.6	248.0 ± 0.0	139.5 ± 0.0	21.7 ± 0.1
	Fababean	3.3 ± 0.0	3.6 ± 0.2	37.5 ± 1.9	129.3 ± 0.4	256.8 ± 0.0	142.1 ± 0.0	49.8 ± 0.2
	Navy bean	4.4 ± 0.1	4.4 ± 0.1	47.0 ± 0.8	175.5 ± 0.7	355.6 ± 0.4	170.6 ± 0.1	42.3 ± 0.1
65-40-11[10]	Pea	5.6 ± 0.1	3.9 ± 0.1	75.1 ± 1.2	154.2 ± 0.4	251.4 ± 0.1	137.9 ± 0.1	44.5 ± 0.2
	Lentil	2.9 ± 0.2	3.4 ± 0.1	38.0 ± 0.6	139.4 ± 0.6	296.8 ± 0.0	148.8 ± 0.7	21.6 ± 0.1
	Fababean	3.3 ± 0.1	3.6 ± 0.1	37.2 ± 0.2	134.7 ± 0.1	284.0 ± 0.4	143.2 ± 1.4	22.7 ± 0.3
	Navy bean	4.1 ± 0.1	4.5 ± 0.1	45.7 ± 0.0	161.9 ± 0.3	373.1 ± 0.0	182.8 ± 0.0	33.6 ± 0.2
Commercial concentrates	Arcon S[11]	5.4 ± 0.0	2.5 ± 0.0	69.6 ± 0.0	132.5 ± 0.1	204.7 ± 0.0	127.8 ± 0.0	47.5 ± 0.3
	Arcon F[12]	3.6 ± 0.0	2.3 ± 0.0	69.5 ± 0.0	147.8 ± 0.0	185.4 ± 0.0	140.5 ± 0.0	14.7 ± 0.2

[1] WHC = Water hydration capacity.
[2] OHC = Oil holding capacity.
[3] EA = Emulsion activity.
[4] ES = Emulsion stability.
[5] FC = Foaming capacity.
[6] FS = Foam stability.
[7] NSI = Nitrogen solubility index.
[8] Mean± standard deviation, n=2.
[9] 52-32-12 = 52% aqueous-alcohol, 32°C, 12-minute extraction time; optimal protein
[10] 65-40-11 = 65% aqueous-alcohol, 40°C, 11-minute extraction time; optimal yield
[11] Arcon S = Commercial soy concentrate.
[12] Arcon F = Commercial soy concentrate.

Table 4.15. Hunter Lab colour values of aqueous-ethanol-washed protein products prepared from air-classified pea, lentil, fababean and navy bean protein fractions using conditions identified as optimal for protein content and yield of pea protein concentrates.

Treatments	Product	L[1]	a[2]	b[3]
Air-classified pea protein	Pea	86.7 ± 0.0[4]	6.3 ± 0.0	21.2 ± 0.0
	Lentil	84.6 ± 0.0	3.6 ± 0.0	16.8 ± 0.0
	Fababean	85.0 ± 0.0	2.5 ± 0.0	14.5 ± 0.0
	Navy bean	89.2 ± 0.1	1.7 ± 1.8	9.7 ± 0.0
52-32-12[5]	Pea	87.0 ± 0.1	0.1 ± 0.0	20.1 ± 0.0
	Lentil	84.9 ± 0.0	0.0 ± 0.0	13.8 ± 0.1
	Fababean	86.9 ± 0.0	0.0 ± 0.0	11.8 ± 0.3
	Navy bean	89.7 ± 0.1	0.1 ± 0.0	9.5 ± 0.0
65-40-11[6]	Pea	89.5 ± 0.0	1.1 ± 0.0	11.8 ± 0.0
	Lentil	86.0 ± 0.0	0.6 ± 0.0	10.1 ± 0.0
	Fababean	88.0 ± 0.7	1.3 ± 1.1	10.6 ± 0.5
	Navy bean	89.5 ± 1.4	1.2 ± 0.2	9.0 ± 1.3

[1] L = 100 white, 0 black.
[2] a = + red, - green.
[3] b = + yellow, - blue.
[4] Mean± standard deviation, n=2.
[5] 52-32-12 = 52% aqueous-alcohol, 32°C, 12-minute extraction time; optimal protein.
[6] 65-40-11 = 65% aqueous-alcohol, 40°C, 11-minute extraction time; optimal yield.

Table 4.16. Oligosaccharide profiles (%, dry basis) of aqueous-ethanol-washed protein products prepared from air-classified pea, lentil, fababean and navy bean protein fractions using conditions identified as optimal for protein content and yield.

Treatments	Product	Raffinose (%)	Stachyose (%)	Verbascose (%)	Total RFO[1]
Air-classified pea protein	Pea	2.4 ± 0.0[2]	4.6 ± 0.0	1.8 ± 0.0	8.8 ± 0.1
	Lentil	1.8 ± 0.0	3.8 ± 0.0	1.0 ± 0.0	6.6 ± 0.1
	Fababean	0.8 ± 0.0	2.6 ± 0.0	0.2 ± 0.0	3.6 ± 0.1
	Navy bean	0.7 ± 0.8	1.9 ± 1.8	0.6 ± 0.1	3.2 ± 0.1
52-32-12[3]	Pea	0.1 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
	Lentil	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
	Fababean	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
	Navy bean	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
65-40-11[4]	Pea	0.3 ± 0.0	1.2 ± 0.0	0.7 ± 0.0	2.2 ± 0.1
	Lentil	0.1 ± 0.0	0.6 ± 0.0	0.3 ± 0.4	1.0 ± 0.1
	Fababean	0.3 ± 0.3	1.3 ± 1.1	0.6 ± 0.1	2.2 ± 0.1
	Navy bean	0.3 ± 0.0	1.2 ± 0.2	0.2 ± 0.0	1.7 ± 0.1

[1] RFO = Raffinose family oligosaccharides.
[2] Mean± standard deviation, n=2.
[3] 52-32-12 = 52% aqueous-alcohol, 32°C, 12-minute extraction time; optimal protein.
[4] 65-40-11 = 65% aqueous-alcohol, 40°C, 11-minute extraction time; optimal yield.

4.3 Composition of the extracts obtained from aqueous-ethanol washing of air-classified pea protein fractions

4.3.1 Composition

The compositions of the extracts prepared using conditions identified as optimum for the yield and protein content of pea protein concentrates are presented in Tables 4.17 and 4.18. The extract obtained after aqueous-ethanol washing of flours contained lipid, protein and oligosaccharide components. The extracts prepared under conditions identified previously as optimal for the yield and protein content of pea protein concentrates were similar in composition with respect to protein, crude fat and ash, but the total lipid content was higher for the extract prepared with a lower concentration of aqueous-ethanol.

Table 4.17. Composition (%, dry basis) of the extracts obtained from aqueous-ethanol washing of air-classified pea protein using treatment conditions optimal for protein content and yield of pea protein concentrates.

Treatment	Protein (%)	Crude Fat (%)	Total Lipid (%)	Ash (%)
52-32-12[2]	1.8 ± 0.1[1]	4.8 ± 0.2	7.5 ± 0.1	3.7 ± 0.4
65-40-11[3]	2.1 ± 0.0	5.1 ± 0.1	5.1 ± 0.3	3.9 ± 0.1

[1] Mean± standard deviation, n=2.
[2] 52-32-12 = 52% aqueous-alcohol, 32°C, 12-minute extraction time; optimal protein.
[3] 65-40-11 = 65% aqueous-alcohol, 40°C, 11-minute extraction time; optimal yield.

The oligosaccharide contents of the extracts were similar (Table 4.18). Stachyose was the most abundant oligosaccharide, and raffinose the least abundant. The raffinose, stachyose and verbascose values of the starting material (air-classified pea protein) were 2.3%, 4.4% and 1.7%, respectively, and the values for the corresponding pea protein concentrates prepared at extraction conditions optimal for the protein content and yield of pea protein concentrates were 0.1%, 0.2% and 0.0%, and 0.3%, 1.2% and 0.7%, respectively. All values are expressed on a dry weight basis.

Table 4.18. Oligosaccharide profiles (%, dry basis) of the extracts obtained from aqueous-ethanol washing of air-classified pea protein using treatment conditions optimal for protein content and yield of pea protein concentrates.

Treatment	Raffinose (%)	Stachyose (%)	Verbascose (%)	Total RFO[1]
52-32-12[3]	1.8 ± 0.4[2]	4.5 ± 0.7	2.7 ± 0.5	9.0 ± 0.0
65-40-11[4]	2.4 ± 0.2	4.6 ± 0.3	2.6 ± 0.2	9.6 ± 0.0

[1] RFO = Raffinose family oligosaccharides.
[2] Mean± standard deviation, n=2.
[3] 52-32-12 = 52% aqueous-alcohol, 32°C, 12-minute extraction time; optimal protein.
[4] 65-40-11 = 65% aqueous-alcohol, 40°C, 11-minute extraction time; optimal yield.

4.3.2 Gel electrophoresis of protein fractions

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was used to examine the protein bands in both pea extracts (Figures 4.19 and 4.20). The gels from both treatment combinations reflected the loss of aqueous-ethanol-soluble proteins from air-classified pea protein in the extract upon aqueous-ethanol washing. The starting material

contained proteins in the molecular mass range of 10-95 kDa. The aqueous-ethanol-washed concentrates consisted predominantly of proteins in the range of 17-95 kDa, and were essentially devoid of lower molecular weight proteins. Both extracts were enriched in low molecular mass proteins, particularly in the range of 10 kDa to approximately 20 kDa.

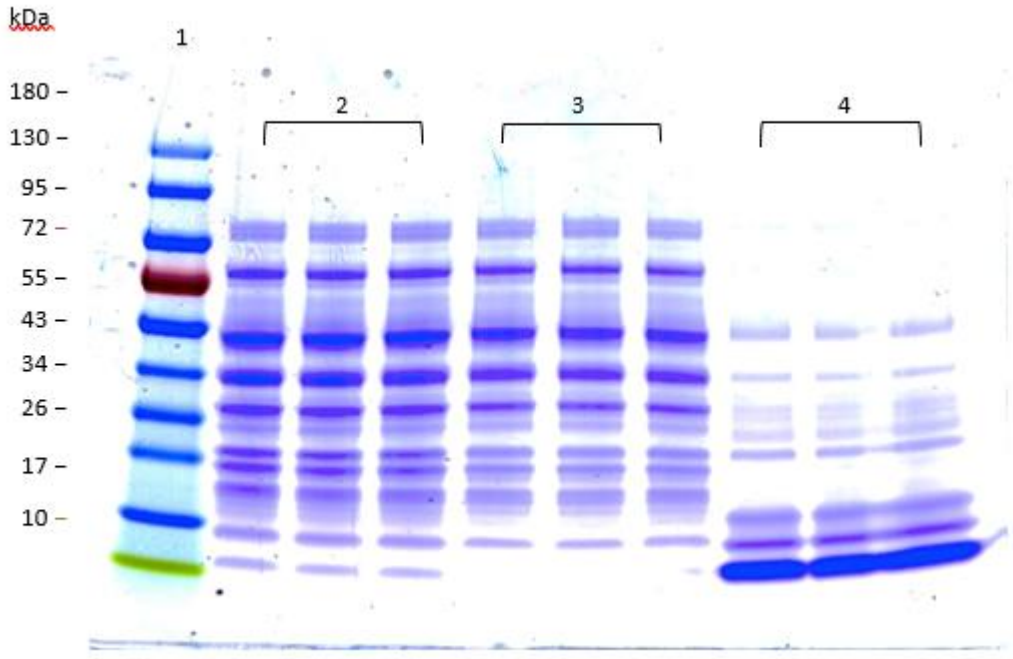

Figure 4.3. SDS-PAGE of pea products. Lanes: (1) molecular weight marker, (2) air-classified pea protein, (3) aqueous-ethanol-washed pea protein concentrate and (4) aqueous-ethanol extract (52% aqueous ethanol, 32°C, 12 minutes).

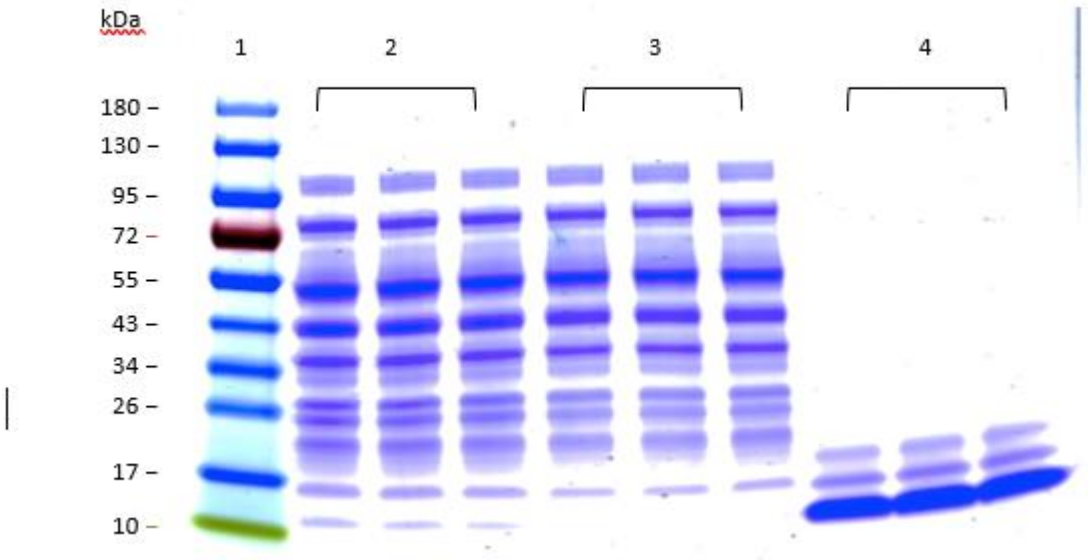

Figure 4.2. SDS-PAGE of pea products. Lanes: (1) molecular weight marker, (2) air-classified pea protein, (3) aqueous-ethanol-washed pea protein concentrate and (4) aqueous-ethanol extract (65% aqueous ethanol, 40°C, 11 minutes).

4.4 Effect of aqueous-ethanol and hexane extraction of pea and chickpea flours prior to air-classification

4.4.1 Composition

Hexane-extracted and 70%-aqueous-ethanol-extracted pea and chickpea flours were analyzed for their protein, starch and crude fat contents, and the values are presented in Table 4.19. All values are expressed on a dry weight basis. All pea flours (unwashed, hexane-washed and aqueous-ethanol-washed) exhibited higher protein contents in comparison to corresponding chickpea flours. Unwashed pea flour and aqueous-ethanol-washed pea flour were similar in protein content and higher in protein than hexane-washed flour. The protein contents of both hexane- and alcohol-washed chickpea flours were slightly higher than that of the unwashed flour.

The starch contents of pea flours washed with hexane or 70%-aqueous-ethanol increased from 51.6% to 54.9% and 53.6%, respectively. A similar and slightly more pronounced effect was observed in the case of chickpea flours, whereas the starch contents in the unwashed flour increased from 45.1% to 52.0% in the hexane-washed flour and 51.1% in the aqueous-ethanol-washed flour.

The crude fat content of unwashed chickpea flour was substantially higher than that of unwashed pea flour (6.3% vs 1.6%). Hexane-washing was more effective in removing crude fat from both pea and chickpea flours compared to 70%-aqueous-ethanol.

Table 4.19. Protein, starch and crude fat (%, dry basis) contents of pea and chickpea flours, before and after hexane or 70%-aqueous-alcohol extraction.

Treatment	Product	Protein (%)	Starch (%)	Crude Fat (%)
Unwashed	Pea	24.7 ± 0.0[1]	51.6 ± 0.3	1.6 ± 0.0
	Chickpea	19.7 ± 0.0	45.1 ± 0.5	6.3 ± 0.2
Hexane-washed	Pea	23.9 ± 0.2	54.9 ± 0.5	0.4 ± 0.1
	Chickpea	20.0 ± 0.1	52.0 ± 0.5	0.5 ± 0.1
Ethanol-washed	Pea	24.7 ± 0.1	53.6 ± 0.1	0.7 ± 0.2
	Chickpea	20.9 ± 0.0	51.1 ± 0.1	1.0 ± 0.2

[1] Mean± standard deviation, n=2.

4.4.2 Fraction yield and composition

The air-classified protein and starch fraction yields for unwashed, hexane-washed and 70%-aqueous-ethanol-washed pea and chickpea flours are presented in Table 4.20. All values are presented on a dry weight basis. Extraction of crude fat from flours prior to air-classification was favourable from the standpoint of the protein content of the fine fraction, but negatively affected the yield of the fine fraction. In the case of pea, air classification of the unwashed flour yielded 46.1% of the fine fraction and 53.9% of the coarse fraction, whereas chickpea yielded 53.9% and 46.1% of the fine and coarse fractions, respectively. Hexane-washed flour yielded 32.6% of the fine fraction and 67.4% of the coarse fraction for pea, whereas chickpea yielded 39.8% and 60.2% of the fine and coarse fractions, respectively. For both pea and chickpea, 70%-ethanol-washed flour produced a higher yield of the coarse fraction in comparison to unwashed flour and hexane-washed flour, and, therefore, a lower yield of the fine fraction. 70%-aqueous-ethanol-washed flour yielded 29.3% of the fine and 70.7% of the coarse fraction for pea, whereas chickpea yielded 31.8% and 68.2% of the fine and coarse fractions, respectively.

For both pea and chickpea, 70%-aqueous-ethanol-washed flours yielded the highest protein contents in both the fine and coarse fractions. For pea, the coarse and fine fraction contained 56.4% protein and 11.2% protein, respectively. For chickpea, corresponding values were 42.4% and

11.6%. In the case of pea, air classification of the unwashed flour resulted in 43.5% protein in the fine fraction and 6.3% protein in the coarse fraction, whereas for chickpea the corresponding fractions contained 28.6% and 11.2% protein. Extraction of pea flour with hexane prior to air-classification resulted in a lower protein content in the coarse fraction. In the case of hexane-washed pea flour, the fine and coarse fractions contained 52.7% protein and 7.0% protein, respectively. For chickpea, the corresponding fractions contained 41.2% and 5.4%.

For both pea and chickpea, unwashed flours exhibited greater starch enrichment in the fine fraction. Unwashed chickpea flour exhibited the lowest starch enrichment in the coarse fraction. Hexane-washed flours had a lower enrichment of starch in the fine fraction when compared to aqueous-ethanol-washed flours. Both pea and chickpea flours exhibited similar values for the starch content of the coarse fraction from 70%-aqueous-ethanol-washed flours.

Table 4.20. Yields and protein contents of starch and protein fractions produced by air classification of hexane-washed or 70%-aqueous-ethanol-washed pea and chickpea flours.

Treatment	Product	Yield (%)		Protein (%)		Starch (%)	
		Fine	Coarse	Fine	Coarse	Fine	Coarse
Unwashed	Pea	46.1 ± 0.0[1]	53.9 ± 2.1	43.5 ± 0.7	6.3 ± 0.1	21.4 ± 3.4	81.2 ± 0.2
	Chickpea	53.9 ± 1.5	46.1 ± 0.3	28.6 ± 3.0	11.2 ± 0.3	27.1 ± 2.5	57.5 ± 1.6
Hexane-washed	Pea	32.6 ± 4.2	67.4 ± 3.5	52.7 ± 0.8	7.0 ± 0.3	8.0 ± 0.4	82.0 ± 3.2
	Chickpea	39.8 ± 3.0	60.2 ± 0.7	41.2 ± 1.2	5.4 ± 0.0	16.8 ± 2.1	76.9 ± 1.3
Ethanol-washed	Pea	29.3 ± 0.0	70.7 ± 0.7	56.4 ± 2.5	11.2 ± 0.5	16.3 ± 0.6	76.2 ± 2.0
	Chickpea	31.8 ± 2.1	68.2 ± 2.1	42.4 ± 2.7	11.6 ± 0.2	26.8 ± 1.0	76.9 ± 1.3

[1] Mean± standard deviation, n=2.

4.5 Evaluation of a product obtained by reflux extraction of air-classified pea protein with 80%-aqueous-ethanol

4.5.1 Compositional analysis

The composition (%, dry basis) of the product resulting from reflux extraction of air-classified pea protein with 80%-aqueous-ethanol (AAFC) and of aqueous-ethanol-washed pea concentrates and commercial soy concentrates are presented in Table 4.21. All values are presented on a dry weight basis. In general, the product was similar in composition to the aqueous-ethanol-washed pea concentrates, but was lower in protein compared to the aqueous-ethanol-washed protein concentrates and the soybean concentrates.

Table 4.21. Composition (% dry weight basis) of the product obtained by reflux extraction of air-classified pea protein with 80% aqueous-ethanol.

Products	Protein (%)	Starch (%)	Crude fat (%)	Total lipid (%)	Ash (%)
AAFC[1]	64.6 ± 0.3[6]	3.1 ± 0.2	1.6 ± 0.1	1.9 ± 0.1	4.7 ± 0.2
52-32-12[2]	73.7 ± 0.4	4.8 ± 0.1	1.7 ± 0.4	4.7 ± 0.3	4.7 ± 0.4
65-40-11[3]	69.2 ± 0.2	4.7 ± 0.2	1.8 ± 0.1	5.0 ± 0.4	4.9 ± 0.2
Arcon S[4]	77.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.0	1.0 ± 0.2	4.3 ± 0.1
Arcon F[5]	70.1 ± 0.4	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	5.9 ± 0.3

[1]AAFC = 80%-aqueous-ethanol, reflux-extracted product from Agriculture and Agri-Food Canada.
[2]52-32-12 = 52% aqueous-alcohol, 32°C, 12 minute extraction time; optimal protein content.
[3]65-40-11 = 65% aqueous-alcohol, 40°C, 11 minute extraction time; optimal yield.
[4]Arcon S = Commercial soy concentrate.
[5]Arcon F = Commercial soy concentrate.
[6] Mean ± standard deviation, n=2.

4.5.2 Functionality

The functionalities of pea protein products and commercial soy concentrates are presented in Table 4.22. In general, the functionalities of the 80%-aqueous-ethanol reflux-extracted product were similar to those of the aqueous-alcohol-washed pea protein concentrates, with the exception of its lower NSI. The 80%-aqueous-ethanol, reflux-extracted product was similar in functionality to one or both of the commercial soy concentrates, with the exception of its higher foaming capacity.

4.5.3 Colour

HunterLab colour parameters (L, a and b values) were determined for the 80%-aqueous-ethanol reflux-extracted pea protein product and compared to those of aqueous-ethanol-washed pea protein concentrates and commercial soy concentrates (Table 4.22). The 80%-aqueous-ethanol reflux-extracted product was whiter, slightly less green and slightly more blue than the pea protein concentrates and the soy controls.

4.5.4 Oligosaccharides

The oligosaccharide contents of the pea protein product prepared by 80%-aqueous-ethanol reflux extraction and of aqueous-ethanol-washed pea concentrates and commercial soy concentrates are presented in Table 4.21. The total oligosaccharide content of the reflux-extracted product was higher than those of the aqueous-ethanol-washed concentrates and similar to that of the air-classified pea protein fraction that contained 2.3% raffinose, 4.4% stachyose and 1.7% verbascose (Tables 4.10 and 4.11). The reflux-extracted product also was higher in oligosaccharides than the commercial soy concentrates. Stachyose was the most abundant oligosaccharide in the reflux-extracted product and in the soy controls.

Table 4.22. Functionality of the product obtained by reflux extraction of air-classified pea protein with 80% aqueous-ethanol.

Product	WHC[1] (mL/g)	OHC[2] (g/g)	EA[3] (%)	ES[4] (%)	FC[5] (%)	FS[6] (%)	NSI[7] (%)
AAFC[9]	2.2 ± 0.1[8]	2.1 ± 0.0	54.0 ± 0.1	101.9 ± 0.2	177.0 ± 1.0	95.5 ± 1.5	16.6 ± 0.1
52-32-12[10]	3.7 ± 0.1	2.1 ± 0.1	55.7 ± 1.3	99.6 ± 2.1	174.0 ± 0.1	97.7 ± 0.1	31.7 ± 0.1
65-40-11[11]	3.9 ± 0.1	2.7 ± 0.1	52.0 ± 1.2	106.7 ± 0.4	174.0 ± 0.1	95.4 ± 0.1	30.8 ± 0.2
Arcon S[12]	4.2 ± 0.0	1.9 ± 0.0	53.7 ± 0.0	102.3 ± 0.1	158.0 ± 0.0	98.7 ± 0.0	36.7 ± 0.3
Arcon F[13]	2.5 ± 0.0	1.6 ± 0.0	48.7 ± 0.0	103.6 ± 0.0	130.0 ± 0.0	98.5 ± 0.0	10.3 ± 0.2

[1] WHC = Water hydration capacity.
[2] OHC = Oil holding capacity.
[3] EA = Emulsion activity.
[4] ES = Emulsion stability.
[5] FC = Foaming capacity.
[6] FS = Foam stability.
[7] NSI = Nitrogen solubility index.
[8] Mean± standard deviation, n=2.
[9] AAFC = 80%-aqueous-ethanol, reflux-extracted product from Agriculture and Agri-Food Canada.
[10] 52-32-12 = 52% aqueous-alcohol, 32°C, 12 minute extraction time; optimal protein content.
[11] 65-40-11 = 65% aqueous-alcohol, 40°C, 11 minute extraction time; optimal yield.
[12] Arcon S = Commercial soy concentrate.
[13] Arcon F = Commercial soy concentrate.

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Table 4.223. Hunter Lab colour values of a product obtained by extraction of air-classified pea protein with 80% aqueous-ethanol and of commercial soy samples.

Product	L[1]	a[2]	b[3]
AAFC[5]	90.3 ± 0.0[4]	-0.65 ± 0.04	10.3 ± 0.0
52-32-12[6]	87.0 ± 0.1	0.1 ± 0.0	20.1 ± 0.0
65-40-11[7]	89.5 ± 0.0	1.1 ± 0.0	11.8 ± 0.0
Arcon S[8]	83.2 ± 0.0	1.35 ± 0.02	16.6 ± 0.0
Arcon F[9]	86.4 ± 0.0	0.72 ± 0.03	14.4 ± 0.1

[1] L = 100 white, 0 black.
[2] a = + red, - green.
[3] b = + yellow, - blue.
[4] Mean± standard deviation, n=2.
[5] AAFC = 80%-aqueous-ethanol, reflux-extracted product from Agriculture and Agri-Food Canada.
[6] 52-32-12 = 52% aqueous-alcohol, 32°C, 12 minute extraction time; optimal protein content.
[7] 65-40-11 = 65% aqueous-alcohol, 40°C, 11 minute extraction time; optimal yield.
[8] Arcon S = Commercial soy concentrate
[9] Arcon F = Commercial soy concentrate.

Table 4.24. Oligosaccharide profiles (%, dry basis) of the product obtained by reflux extraction of air-classified pea protein with 80% aqueous-ethanol and of control samples.

Treatments	Raffinose (%)	Stachyose (%)	Verbascose (%)	Total RFO[1]
AAFC[3]	0.7 ± 0.0[2]	3.8 ± 0.0	3.7 ± 0.0	8.2 ± 0.0
52-32-12[4]	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
65-40-11[5]	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Arcon S[6]	0.2 ± 0.0	0.8 ± 0.0	0.1 ± 0.0	1.1 ± 0.0
Arcon F[7]	0.6 ± 0.0	3.4 ± 0.0	0.2 ± 0.0	4.2 ± 0.0

[1] RFO = Raffinose family oligosaccharides.

[2] Mean± standard deviation, n=2.

[3]AAFC Agriculture and Agri Foods Canada reflux-extracted product.

[4]52-32-12 = 52% aqueous-alcohol, 32°C, 12 minute extraction time; optimal protein content.

[5]65-40-11 = 65% aqueous-alcohol, 40°C, 11 minute extraction time; optimal yield.

[6]Arcon S = Commercial soy concentrate.

[7]Arcon F = Commercial soy concentrate.

5. DISCUSSION

5.1 Yield, composition and functionality of alcohol-washed protein concentrates prepared from air-classified pea protein

5.1.1 Yield and composition

In a solid-liquid extraction process, a solid is contacted with a liquid in which one or several components (solutes) are soluble. Solutes may differ in their solubility in the solvent. When a solid is brought into contact with a liquid, mass transfer of components between phases occurs until a state of equilibrium is achieved (Brennan, 2011). Several parameters affect the rate of extraction, including: i) solid-liquid interface contact area; ii) concentration gradient; iii) contact time; and iv) extraction temperature.

Optimization of an aqueous-alcohol washing process for the production of protein concentrates from air-classified pulse flours is complex. Three variables, namely the concentration of alcohol (ethanol or isopropanol), temperature of extraction, and time of extraction were assumed to bear an effect on the yield and protein content of the concentrates produced. The present study demonstrated the usefulness of a Box-Behnken design for optimization of the aqueous-alcohol washing process and provided clear evidence that optimal conditions with respect to concentrate yield and protein content could be identified.

Alcohol concentration proved to bear a significant effect on both the yield and protein content of protein concentrates, but equally relevant was the observation that extraction temperature and extraction time had no significant impact on the dependent variables. This was attributed to the fine particle size of the starting material that provided a large surface area for extraction and a short distance for solvent penetration and mass transfer, without the need of intervention, i.e. elevated temperature or extended extraction time. The derived polynomial equations aided in predicting the values of selected independent variables for identification of optimum treatment combinations. The model predicted, with high precision, protein content and

product yield values for aqueous-isopropanol-washed concentrates. In the case of aqueous-ethanol-washed products, the model was able to predict values for concentrate yield only. For statistical analysis of protein content values for aqueous-ethanol-washed concentrates, the model should be used with caution but could be considered to reflect response tendencies.

This study showed that protein concentrates higher in protein content could be prepared using lower concentrations of aqueous-alcohol, whereas a higher aqueous-alcohol concentration favoured production of concentrates in higher yield, i.e. the concentrate yield increased and the protein content decreased for all concentrates as the concentration of alcohol in the extraction solvent increased. This was attributed to a decrease in the solubility of non-protein constituents as the alcohol concentration increased. Results were analogous to those reported by Pokatong (1994). In that study, protein concentrates were prepared from air-classified pea protein and soy flour containing 58.1% and 55.9% protein, respectively, using aqueous-alcohol washing, acid washing or alkali extraction. Although a similar effect of alcohol concentration on yields of protein concentrates was reported by Pokatong (1994), the yields of concentrates prepared by all three processing methods exceeded the yields obtained in the current study. The protein contents of the concentrates prepared by all three processing methods from pea and soy were similar to the protein contents of protein concentrates prepared in the current study, although the protein contents were aligned more closely with those of the alcohol-washed concentrates. This was attributed to differences in the processing methods, i.e. differences in the solubility of protein and non-protein constituents in aqueous alcohol, alkali and acid, and to inherent differences in the composition of soy flour and air-classified pea protein. The protein contents of concentrates prepared in the current study also were similar to those of commercial soy protein concentrates, Arcon S and Arcon F, that contained 77.2% and 70.1% protein, respectively.

The starch contents of all concentrates were higher, whereas the crude fat and total lipid contents were reduced, compared to corresponding values for the starting material. A more pronounced effect of fat removal was observed in isopropanol-washed concentrates, which was attributed to the lower polarity of isopropanol (Nollet, 2004).

5.1.2 Functionality

The protein concentrates exhibited higher functionality values compared to the starting material for all attributes assessed, with the exception of foaming capacity and nitrogen solubility index. In many respects, functionalities were comparable to those of the commercial concentrates (Arcon S and Arcon F). Schwenke et al. (1983) found that the foaming capacity and foam stability of fababean isolates could be improved by treatment with isopropanol or moderate temperature. Toews & Wang (2013) found a positive correlation between foaming capacity and the protein content of protein concentrates prepared from several pulses, and a negative correlation with fat content.

Despite the lower crude fat and total lipid contents of aqueous-isopropanol-washed concentrates, their foaming capacities were lower compared to those of ethanol-washed concentrates. The reason for this behaviour is unknown. Adebiyi & Aluko (2011) established that water-soluble proteins produced the highest foam volume. King et al. (1985) and Sikorski (2001) considered polarity and hydrophobicity to be the determining factors of the emulsifying and foaming properties of protein. It is possible that the loss of water-soluble proteins, or of proteins of lower polarity or greater hydrophobicity during aqueous-isopropanol extraction of air-classified pea protein, was responsible for the lower foaming capacity values of isopropanol-washed concentrates. Functionality values for both alcohol-washed concentrates were calculated on a per gram of protein basis (Tables 4.5 and 4.7) for comparison with values determined on a per gram of sample basis (Tables 4.4 and 4.6).

The protein concentrates were washed with 95%-aqueous-alcohol prior to their being dispersion-dried. This step was employed to reduce the water content of the samples prior to drying, thereby facilitating the drying step and reducing lumping/caking of the dried products. The 95%-aqueous-alcohol treatment may have impacted the functionality of the protein concentrates, to the extent that differences in functionality among concentrates prepared by the various treatments were less than would have been observed had the 95%-aqueous-alcohol washing step not been employed. This said, distinct differences in functionality among the concentrates were observed.

Aqueous-isopropanol-washed concentrates were whiter and less yellow than aqueous-ethanol-washed products, indicating greater solubility of coloured compounds in aqueous-isopropanol than in aqueous-ethanol. It was determined informally that the aqueous-alcohol-

washed protein concentrates were essentially devoid of flavour or odour, reflecting the solubility of flavour and odour components in aqueous-alcohol. Aqueous-alcohol-washed samples exhibited reduced oligosaccharide contents compared to the starting material. The predominant oligosaccharides identified were raffinose, stachyose and verbascose, with stachyose being the most abundant. It was found that when a lower concentration of alcohol was employed for extraction, the oligosaccharide contents were lower in the resultant alcohol-washed concentrates. This would be indicative of the higher solubility of oligosaccharides in water than in alcohol. Higher yields of raffinose family oligosaccharides (RFOs) at lower ethanol concentrations in the extraction solvent also have been reported in earlier studies (Shukla, 1987; Kosson, 1992; Johansen et al., 1996). Knudsen & Li (1991) recommended water as the optimal solvent for extraction of RFOs from plant material. However, Johansen et al. (1996) stated that such a medium also would facilitate interference between soluble carbohydrates and other water-soluble substances, such as some dietary fibre polysaccharides and proteins. The effectiveness of 50%- aqueous-ethanol as an extraction solvent for RFOs can be ascribed to the higher polarity of a lower concentration of ethanol Ekvall et al. (2007).

Due to the small differences in composition and functionality between corresponding aqueous-isopropanol-washed and aqueous-ethanol-washed products, aqueous-ethanol was chosen as the solvent for use in later studies.

5.2 Yield, composition and functionality of alcohol-washed protein concentrates prepared from air-classified lentil, fababean and navy bean protein

The feasibility of producing aqueous-ethanol-washed protein concentrates containing more than 65% protein on a moisture-free basis, and having functionality similar to that of aqueous-alcohol-washed concentrates from pea and commercial soy protein concentrates, from a variety of air-classified pulse protein fractions was demonstrated. Aqueous-alcohol-washed concentrates prepared from pea and fababean contained 65-70% protein, whereas those prepared from lentil and navy bean did not, which reflected the lower levels of protein and higher levels of starch in the air-classified lentil and navy bean protein fractions. As observed for aqueous-ethanol-washed concentrates prepared from pea, concentrates prepared from lentil, fababean and navy bean were higher in starch and ash, and lower in crude fat and total lipid, than the starting materials, due to

the removal of alcohol-soluble constitutents. Boye et al. (2010) prepared protein isolates, using isoelectric precipitation (IEP) and ultrafiltration (UF), from yellow pea, chickpea (Desi and Kabuli) and lentil (red and green) flours containing 21.09%, 20.52%, 16.71%, 25.88% and 23.03% protein, respectively. It was observed that red lentil flour, having the highest protein content, yielded isolates with higher protein concentrations, 78.2% and 82.7% using IEP and UF, respectively.

In general, aqueous-ethanol-washed products exhibited higher or similar functionality values as compared to the corresponding starting materials, although the water holding capacity and oil holding capacity values of all products were comparable, and emulsion activity values for aqueous-ethanol-washed products prepared from lentil, fababean and navy bean were lower than those of the respective starting materials. Several researchers have suggested that the solubility and surface charge of protein play a critical role in its emulsification properties (Karaca et al., 2011; Meng & Ma, 2002; Mundi & Aluko, 2012; Ragab et al., 2004; Withana-Gamage et al., 2011; Yin et al., 2010; Zhang et al., 2009). Zhang et al. (2009) suggested that the emulsifying activity of proteins was greatly dependent on their flexibility. It is conceivable that the aqueous-ethanol washing process affected the protein molecular conformation in a way that enhanced the emulsifying properties of protein concentrates from pea, but had no effect for protein concentrates from lentil, fababean and bean (Toews & Wang, 2013). An interesting anamoly was that aqueous-ethanol washing had a negative effect on foaming capacity for all pulses, with the exception of bean despite its low protein content. It is possible that the low fat content of the bean concentrate positively affected its foaming properties. (Toews & Wang (2013) also reported the highest foaming capacity for navy bean samples compared to all other pulse protein isolates prepared in their study. Aqueous-ethanol washing had a significant negative impact on the nitrogen solubility index of pulse protein concentrates. A similar effect has been reported by others (Pokatong, 1994). Boye et al. (2010) attributed differences in the functionalities of protein isolates prepared from several pulses to the variety and extraction process employed.

Aqueous-ethanol-washed concentrates were whiter and less yellow than the starting air-classified fractions used in their preparation indicating greater solubility of soluble coloured compounds in aqueous-ethanol. The observed differences in the colour characteristics of different protein concentrates could be attributed to differences in coloured pigments in the pulses (Toews & Wang, 2013). Aqueous-alcohol-washed samples exhibited reduced oligosaccharide contents

compared to the starting material. The concentrates were somewhat similar in composition and functionality with commercial concentrates (Arcon S and Arcon F) with inherent differences contributed by differences in the type of pulse and the methods used for extraction.

Functionality values for aqueous-alcohol-washed (ethanol and isopropanol) pea concentrates were calculated on a per gram of protein basis (Tables 4.5 and 4.7) for comparison with values determined on a per gram of sample basis (Tables 4.4 and 4.6).

Functionality values for aqueous-ethanol-washed concentrates from pea, lentil, fababean and navy bean were calculated on a per gram of protein basis (Table 4.14) for comparison with values determined on a per gram of sample basis (Table 4.13).

5.3 Composition of the extracts obtained from aqueous-ethanol washing of air-classified pea protein fraction

The extracts obtained by aqueous-ethanol washing of flours contained lipid, protein and oligosaccharide components soluble in aqueous-ethanol at the alcohol concentrations employed (Table 4.17). Compositionaly, the extracts were similar, with some exceptions. The extract prepared using conditions identified as optimal for the protein content of pea protein concentrates exhibited a higher total lipid content. The Osborne fractionation classifies proteins into water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamins, and acid/alkali-soluble glutelins (Osborne & Campbell, 1898). The main components of legume storage proteins are globulins and some albumins, and prolamins and glutelins are detected in small amounts (Martínez et al., 2007).

The SDS-PAGE gels (Figures 4.3 and 4.4) from both treatment combinations reflected the loss of aqueous-ethanol-soluble proteins from air-classified pea protein in the extracts. Electrophoresis of proteins from air-classified pea protein under reducing conditions showed bands ranging from 99 to 11.8 kDa that originated mainly from legumin and vicilin, which are 11S and 7S globulins, respectively (Shand et al., 2007). Air-classified pea protein contained ten proteins within the molecular mass range of 10-95 kDa. The aqueous-ethanol-washed concentrates consisted predominantly of proteins within the range of 17-95 kDa, with 55 kDa and 43 kDa proteins in the highest proportion, and were devoid of lower molecular mass peptides. Both extracts were enriched in low molecular mass proteins, particularly in the range of 10 kDa to approximately

20 kDa. Cre´vieu et al. (1997) identified a protein with an apparent molecular mass of 90 kDa in pea and soy samples to be lipoxygenase. Legumin, a hexameric protein, dissociates into two subunit peptides (a: acidic, 38-40 kDa and b: basic, 19-22 kDa) when S–S bonds are broken under reducing conditions (Bacon et al., 1989; Cre´vieu et al., 1997; Casey, 1979; Gatehouse et al., 1980; Gatehouse et al., 1982; Matta et al., 1981; Shand et al., 2007). Vicilin is a trimeric protein composed of three heterogeneous subunits of 50 and 70 kDa. No S–S bonds are involved in stabilizing the vicilin protein structure (O'Kane et al., 2004a; Shand et al., 2007). Gatehouse et al. (1982) indicated that the major polypeptides of the vicilin fraction (7S) have molecular masses of 71, 50 and 33 kDa, with minor components of 19-12.5 kDa. The 70 kDa protein band has been considered to be convicilin; however, the detailed work of O'Kane et al. (2004a) demonstrated that it is the a-subunit of vicilin (Shand et al., 2007).

5.4 Effect of aqueous-ethanol and hexane extraction of pea and chickpea flours prior to air-classification

Sosulski & Youngs (1979) found that chickpea flour exhibited poor separation during air classification compared to pea, bean and lentil, and attributed the poor separation to the higher fat content of chickpea flour. This study conclusively demonstrated that extraction of crude fat from chickpea flour prior to air classification improved the separation of coarse and fine fractions (Table 4.20). Hexane was somewhat more effective in extracting fat from pea and chickpea flours than was 70%-aqueous-ethanol, due to its lower polarity.

For both pea and chickpea, extraction of crude fat prior to air classification reduced the yield but increased the protein content of the fine fraction. This was attributed to reduced agglomeration of starch and protein particles in flours that had been extracted with either hexane or 70%-aqueous-ethanol, and reduced sticking of defatted flours to the internal components of the pin mill and air-classifier (Pelgrom et al., 2013; Dijkink et al., 2007; Tyler et al., 1981; Tyler & Panchuk, 1982). The result was superior separation of starch and protein during air classification. The use of a larger cut-size would have enhanced the yield of the fine fraction, but would have reduced the protein contents of both the coarse and fine fractions (Pelgrom, Boom, & Schutyser, 2015; Pelgrom et al., 2013; Tyler et al., 1981; Tyler & Panchuk, 1982). The impact of extraction of crude fat on fraction yields was similar for the two solvents and for both pea and chickpea, despite the lower initial crude fat content of pea flour. Extraction of crude fat with hexane resulted in lower protein contents

in the coarse fractions from both pea and chickpea, and lower levels of starch in the fine fraction, as compared to extraction with 70%-aqueous-ethanol. Differences in the effects of extraction of fat with hexane vs. 70%-aqueous alcohol would reflect the solubility of some protein, polar lipid and oligosaccharides in 70%-aqueous-alcohol and their insolubility in hexane. Chickpea starch granules are smaller than those of pea. The smaller difference in size between starch granules and protein particles in chickpea would result in reduced separation sharpness and higher starch contents in chickpea protein fractions (Pelgrom et al., 2015; Pelgrom et al., 2013; Tyler et al., 1981; Tyler & Panchuk, 1982).

Clearly, the yield and composition of air-classified fractions are dependent on a complex interplay among the composition (particularly crude fat and protein contents) of the starting material, starch granule size and the cut-size of the air-classifier. Unfortunately, the benefits of extraction of crude fat from flours prior to air-classification likely would not be attainable commercially for economic reasons.

5.5 Evaluation of a product obtained by reflux extraction of air-classified pea protein with 80%-aqueous-ethanol

The product resulting from 80%-aqueous-ethanol reflux extraction of air-classified pea protein was similar in composition and functionality to aqueous-alcohol-washed pea protein concentrates and soy controls (Tables 4.21-4.24). The main exceptions were the lower protein and total lipid contents, brighter colour, and higher oligosaccharide content of the reflux-extracted product compared to aqueous-alcohol-washed products. The reflux-extracted product barely met the level of protein required for a protein concentrate (>65% protein on a dry weight basis). The higher temperature (~80°C) and ethanol concentration employed in reflux extraction clearly did not have a marked effect on the composition or functionality of the protein concentrate, indicating that a concentrate prepared from air-classified pea protein could substitute in applications currently employing soy concentrate.

6. SUMMARY AND CONCLUSIONS

A Box Behnken design with three factors, three levels and 13 treatment combinations with replicated centre-point treatments was used to describe quantitatively the relationship between aqueous-alcohol concentration, extraction temperature and extraction time and the protein contents and yields of pea protein concentrates. Both aqueous-ethanol and aqueous-isopropanol were evaluated, and similar results were obtained for the two alcohols.

Alcohol concentration was found to bear a significant effect on both the yield and protein content of protein concentrates. The study demonstrated that a lower concentration of alcohol was more favourable in preparing protein concentrates with higher protein content, whereas a higher aqueous-alcohol concentration favoured production of concentrates in higher yield. In other words, concentrate yields increased and protein contents decreased for all concentrates as the concentration of alcohol in the extraction solvent increased. Time and temperature were found to have no significant effect on protein content or yield. This could be attributed to the fine particle size and large surface area of air-classified protein fractions. The model was able to predict protein content and product yield values for aqueous-isopropanol-washed concentrates with high precision. However, for aqueous-ethanol-washed products, the model was able to predict values for concentrate yield only; hence, optimal conditions for yield were determined by canonical and ridge analysis. Optimal conditions for protein content were identified as 52% aqueous-ethanol, 32°C, 12-minute extraction time or 55% aqueous-isopropanol, 50°C, 11-minute extraction time. Optimal conditions for yield were identified as 65% aqueous-ethanol, 40°C, 11-minute extraction time or 70% aqueous-isopropanol, 44°C, 10-minute extraction time.

All concentrates were true protein concentrates (protein concentration >65% on a dry weight basis) and exhibited substantially higher protein contents than the starting material. Increasing concentrations of alcohol negatively affected the protein contents of the concentrates. Aqueous-ethanol-washed and aqueous-isopropanol-washed concentrates exhibited higher starch contents than the starting material, air-classified pea protein. The concentrations of crude fat and total lipid

in protein concentrates were found to be lower than those of the starting material, more so in the case of aqueous-isopropanol-washed concentrates since aqueous-isopropanol was found to be more effective than aqueous-ethanol for all corresponding treatment combinations in the removal of crude fat and total lipid.

The concentrates also were evaluated for their functionality and compared with commercial soy protein concentrates. Water holding capacity (WHC) and oil holding capacity (OHC) were similar for all pea concentrates. Neither the alcohol used, i.e. ethanol or isopropanol, nor the concentration of aqueous-alcohol was found to have any effect on these functional properties. All concentrates exhibited higher WHCs in comparison to the starting material. Emulsion activity (EA) values were similar for all concentrates prepared in this study, and protein concentrates exhibited improved EAs compared to the starting material and were similar in EA to commercial concentrates. The emulsion stability (ES) of the sample prepared with 50%-aqueous-ethanol at 20°C and an extraction time of 10 minutes was similar to that of the starting material. All other concentrates exhibited ESs higher than that of the starting material.

The protein concentrates prepared by aqueous-ethanol washing exhibited similar foaming capacities (FCs) for all treatment combinations, and values were similar to that of the starting material. Aqueous-isopropanol-washed concentrates had markedly lower FCs than the aqueous-ethanol-washed concentrates and the starting material. Foam stability (FS) was found to increase with an increase in aqueous-alcohol concentration. Nearly all of the alcohol-washed concentrates had somewhat higher FS than did the starting material.

All treatments negatively affected nitrogen solubility (NSI). Aqueous-ethanol and aqueous-isopropanol had similar effects. Extraction temperature also had a detrimental effect on the NSIs of the protein concentrates.

The alcohol-washed concentrates became whiter, less red and less yellow as the concentration of aqueous-alcohol increased. Aqueous-isopropanol-washed concentrates were whiter and less yellow than aqueous-ethanol-washed products, thus indicating greater solubility of coloured compounds in aqueous-isopropanol than in aqueous-ethanol.

The concentrates exhibited markedly lower raffinose-family oligosaccharide (RFO) contents compared to the starting material. Aqueous-isopropanol-washed concentrates were found to be

somewhat more depleted in oligosaccharides than were corresponding aqueous-ethanol-washed concentrates. Among the oligosaccharides identified, stachyose was the most abundant. It also was found that with a lower concentration of alcohol in the extraction solvent, the oligosaccharide content was lower in the resultant alcohol-washed concentrate, indicating the higher solubility of RFOs in water than in alcohol.

The study demonstrated the usefulness of a Box-Behnken design for optimization of the aqueous-alcohol washing process and provided clear evidence that optimal conditions with respect to concentrate yield and protein content could be identified.

The optimal extraction conditions identified for the yield and protein content of aqueous-ethanol-washed pea protein concentrates were used to prepare protein concentrates from air-classified fababean, lentil and navy bean protein fractions. Only products prepared from fababean contained over 65% protein on a dry weight basis and could be classified as true protein concentrates. In the case of lentil and navy bean products, the low protein concentrations reflected the lower levels of protein and higher levels of starch in the air-classified lentil and navy bean protein fractions. The optimal conditions identified for the yield of pea protein concentrates (65% aqueous ethanol, 40°C, 11 minutes) produced higher product yields from fababean, lentil and navy bean compared to conditions identified as optimal for the protein content of pea protein concentrates (52% aqueous ethanol, 32°C, 12 minutes).

As observed for aqueous-ethanol-washed concentrates prepared from pea, aqueous-ethanol-washed products prepared from fababean, lentil and navy bean were higher in starch and ash, and lower in crude fat and total lipid, than the starting materials due to the removal of aqueous-ethanol-soluble constitutents. Lentil products exhibited the highest starch contents, and pea the lowest. The fababean concentrate prepared using 65%-aqueous-ethanol, 40°C and 11 minutes exhibited the highest increase in starch content upon aqueous-ethanol washing. All concentrates were somewhat depleted in their crude fat, total lipid and ash contents upon aqueous-ethanol washing.

Functionalities of fababean, lentil and navy bean concentrates also were tested and compared with those of concentrated protein products from pea. Products prepared from air-classified pea protein exhibited higher WHCs than did those from fababean, lentil or navy bean when prepared using conditions identified as optimal for protein content or yield of pea protein

concentrates. The WHCs of the products from fababean, lentil and navy bean were similar. The WHCs of pea protein concentrates were similar to that of one of the commercial soy concentrates, as were values for the fababean, lentil and navy bean products. All protein products, including both of the commercial soy concentrates, exhibited similar OHCs. The concentrates prepared from pea exhibited the highest EA values, similar to those of the commercial soy concentrates. The EA values for the fababean, lentil and navy bean products were similar. Pea protein concentrates exhibited the highest ES values. The FCs of all of the aqueous-ethanol-washed products were higher than those of the commercial soy concentrates. As observed in the earlier study, all aqueous-ethanol-washed products exhibited much lower NSIs than did the corresponding starting materials.

The aqueous-ethanol-washed products were whiter, less red and less yellow than the respective starting materials. Loss of colour was more evident in samples prepared using extraction conditions identified as optimal for the yield of pea protein concentrates.

RFO analysis revealed that the extraction conditions identified as optimal for the protein content of pea protein concentrates were more effective in extracting oligosaccharides from air-classified protein fractions than were the conditions identified as optimal for yield. Stachyose was found to be the most abundant oligosaccharide.

The concentrates were found to be somewhat similar in composition and functionality to commercial soy concentrates, with inherent differences contributed by differences in the type of pulse and the conditions used for extraction. This study demonstrated that it was indeed possible to prepare protein concentrates that contained over 65% protein using aqueous-ethanol washing from other air-classified pulse protein fractions. However, also crucial was the observation that the preparation of true protein concentrates was dependent on the protein content and the content of alcohol extractables in the starting material.

The extracts obtained as by-products of the preparation of protein concentrates from air-classified pea protein at conditions identified as optimal for protein content and yield were analyzed for their. composition. The extracts were similar with respect to their protein, crude fat and ash contents, but the total lipid content was higher for the extract prepared with a lower concentration of aqueous-ethanol. Stachyose was found to be the most abundant oligosaccharide, and raffinose the least abundant.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions was used to examine the protein constituents in air-classified pea protein, aqueous-ethanol-washed protein, and extracts. The starting material contained proteins in the molecular mass range of 10-95 kDa. The aqueous-ethanol-washed concentrates consisted predominantly of proteins in the range of 17-95 kDa. The extracts were found to be enriched in low molecular mass proteins, particularly in the range of 10 kDa to approximately 20 kDa. The reduced levels of low molecular weight proteins in the aqueous-ethanol-washed concentrates was in keeping with their enrichment in the extracts. This study demonstrated the solubility in aqueous-ethanol of crude fat and lipid components in air-classified pea protein, and the resultant increase in the concentration of protein in protein concentrates post aqueous-ethanol washing. It also was successful in demonstrating the solubility of low molecular proteins in aqueous-alcohol as evidenced through their enrichment in the extract. However, the potential usefulness of the extract is unknown and requires further investigation.

Pea and chickpea flours were washed with 70%-aqueous-ethanol or hexane prior to fine milling and air classification to confirm whether the relatively poor separation efficiency of starch and protein fractions associated with chickpea was due to its inherent higher fat content. All pea flours (unwashed, hexane-washed and aqueous-ethanol-washed) exhibited higher protein and starch contents in comparison to corresponding chickpea flours. Unwashed pea flour and aqueous-ethanol-washed pea flour were similar in protein content and higher in protein than hexane-washed pea flour. The protein contents of both hexane-washed and alcohol-washed chickpea flours were slightly higher than that of unwashed chickpea flour.

The crude fat content of unwashed chickpea flour was substantially higher than that of unwashed pea flour (6.3% vs 1.6%). Hexane was found to be more effective in removing crude fat from both pea and chickpea flours compared to 70%-aqueous-ethanol. Extraction of crude fat from flours prior to air-classification was deemed favourable from the standpoint of the protein content of the fine fraction; however, it was detrimental to the yield of the fine fraction.

The composition and functionality of a protein product prepared from air-classified pea protein by reflux extraction with 80%-aqueous-ethanol was compared to the composition and functionality of pea protein concentrates prepared by aqueous-ethanol extraction and of commercial soy concentrates. It was found that the product was similar in composition to aqueous-

ethanol-washed pea concentrates and soy concentrates, except that it was lower in protein and higher in RFOs. In general, the functionalities of the 80%-aqueous-ethanol reflux-extracted product were similar to those of aqueous-ethanol-washed pea protein concentrates, with the exception of its lower NSI. The 80%-aqueous-ethanol reflux-extracted product was similar in functionality to one or both of the commercial soy concentrates, with the exception of its higher foaming capacity. The reflux-extracted product also was whiter, slightly less green and slightly more blue than aqueous-ethanol-washed pea protein concentrates and the soy controls.

Further research in this area could include: i) compositional and functionality studies on aqueous-alcohol-washed protein concentrates prepared from air-classified pulse protein fractions containing higher levels of protein: ii) more detailed evaluation of the composition of air-classified protein fractions, aqueous-alcohol-washed protein concentrates and aqueous-alcohol extracts, particularly with respect to their dietary fibre contents; iii) sensory and storage stability/shelf-life studies on aqueous-alcohol-washed pulse protein concentrates; iv) preparation of aqueous-alcohol-washed pulse protein concentrates at pilot scale which would facilitate end-product functionality studies; and v) preparation of aqueous-alcohol-washed pulse protein concentrates without the use of a final wash with 95%-aqueous-alcohol, which may result in enhanced differences in functionality among concentrates prepared using various concentrations of aqueous alcohol.

7. BIBLIOGRAPHY

Abbey, B., & Ibeh, G. (1988). Functional properties of raw and heat processed cowpea (*Vigna unguiculata , Walp*) flour. *Journal of Food Science,53*(6), 1775-1777.

Adebiyi, & Aluko. (2011). Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum L*.) seed protein isolate. *Food Chemistry, 128*(4), 902-908.

Agboola, Mofolasayo, Watts, & Aluko. (2010). Functional properties of yellow field pea (*Pisum sativum L*.) seed flours and the in vitro bioactive properties of their polyphenols. *Food Research International, 43*(2), 582-588.

Akpinar, Ali Akpinar, & Türkoğlu. (2001). Total lipid content and fatty acid composition of the seeds of some *Vicia* L. species. *Food Chemistry, 74*(4), 449-453.

Alibhai, Mondor, Moresoli, Ippersiel, & Lamarche. (2006). Production of soy protein concentrates/isolates: Traditional and membrane technologies. *Desalination, 191*(1), 351-358.

American Association of Cereal Chemists. (1995). *Approved methods of the American Association of Cereal Chemists* (9th ed.). St. Paul, Minn., USA: American Association of Cereal Chemists.

Anonymous. (1992). *Technology of production of edible flours and protein products from soybean*. Retrieved 2012, from FAO Corporate Document Repository: http://www.fao.org/docrep/t0532e/t0532e06.htm

Arora, S. K. (1983). Legume carbohydrates. In *Chemistry and Biochemistry of legumes* (pp. 1-50). London: Edward Arnold.

Bacon, J., Noel, T., & Wright, D. (1989). Studies on the thermal behaviour of pea (*Pisum sativum*) vicilin. *Journal of the Science of Food and Agriculture, 49*(3), 335-345.

Baker, E. C., Mustakas, G. A., & Warner, K. (1979). Extraction of Defatted Soybean Flours and Flakes with Aqueous Alcohols: Evaluation of Flavor and Selected Properties. *Journal of Agricultural and Food Chemistry, 27*(5), 969-973.

Bergthaller, W., B. H. Dijkink, H. C. Langelaan, and J. M. Vereijken. 2001. Legume protein preparation and functional properties. *Food* 45:390-392.

Berrios, Morales, Cámara, & Sánchez-Mata. (2010). Carbohydrate composition of raw and extruded pulse flours. *Food Research International, 43*(2), 531-536.

Boye, Zare, & Pletch. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414-431.

Brennan, J., Grandison, Alistair S, & Wiley InterScience. (2011). *Food processing handbook* (2nd ed.). Weinheim: Wiley-VCH.

Butt, M. S., & Batool, R. (2010). Nutritional and functional properties of some promising legumes protein isolates. *Pakistan Journal of Nutrition, 9*(4), 373-379.

Campbell, & Mougeot. (1999). Creation and characterisation of aerated food products. *Trends in Food Science & Technology, 10*(9), 283-296.

Casey, R. Immunoaffinity chromatography as a means of purifying legumin from *Pisum* (peas) seeds. *Biochemistry Journal*, 177 (1979), pp. 509-520

Casey, R. (1982). The genetics of pea seed storage proteins. *Plant Foods for Human Nutrition,31*(3), 281-295.

Chakraborty, P. (1986). Coconut protein isolate by ultrafiltration. In: M. LeMeguer and P. Jelen, Editors,*Food Engineering and Process Applications Elsevier Applied Science Publishers*, *New York,* 2 : 308–315.

Chau, C., Cheung, P., & Wong, Y. (1997). Effects of cooking on content of amino acids and antinutrients in three Chinese indigenous legume seeds. *Journal of the Science of Food and Agriculture,75*(4), 447-452.

Chavan, Mckenzie, & Shahidi. (2001). Functional properties of protein isolates from beach pea (Lathyrus maritimus L.). *Food Chemistry, 74*(2), 177-187.

Chibbar, R., Ambigaipalan, P., & Hoover, R. (2010). REVIEW: Molecular Diversity in Pulse Seed Starch and Complex Carbohydrates and Its Role in Human Nutrition and Health. *Cereal Chemistry, 87*(4), 342-352.

Chou, D., & Morr, C. (1979). Protein-water interactions and functional properties. *Journal of the American Oil Chemists' Society, 56*(1), A53-A62.

Chrismon, R. L. (1978). In *Air classification in resource recovery* (pp. 2). Washington: National Centre for Resource Recovery.

Chung, K., Wong, T., Wei, C., Huang, Y., & Lin, Y. (1998). Tannins and Human Health: A Review. *Critical Reviews in Food Science and Nutrition, 38*(6), 421-464.

Cornec, Cho, & Narsimhan. (1999). Adsorption Dynamics of α-Lactalbumin and β-Lactoglobulin at Air–Water Interfaces. *Journal of Colloid and Interface Science, 214*(2), 129-142.

Crévieu, I. M., Carré, B., Chagneau, A., Quillien, L., Guéguen, J., & Bérot, S. (1997). Identification of Resistant Pea (*Pisum sativum L.*) Proteins in the Digestive Tract of Chickens. *Journal of Agricultural and Food Chemistry, 45*(4), 1295-1300.

Dalgetty, D. D., Baik, B.-K., & Swangson, B. G. (2003). Pulses. In *Encyclopedia of food sciences and nutrition (Second Edition)* (pp. 4880-4887). Washington: Elsevier Science Ltd.

Damodaran, S., Parkin, Kirk L., & Fennema, Owen R. (2008). *Fennema's food chemistry* (4th ed., Food science and technology (Taylor & Francis)). Boca Raton: CRC Press/Taylor & Francis

Damodaran, S. (2005). Protein stabilization of emulsions and foams. *Journal of Food Science, 70*(3), R54-R66.

Dickinson, E. (1992). *An introduction to food colloids*. Oxford [England]; New York: Oxford University Press.

Dickinson, E. (1999). Adsorbed protein layers at fluid interfaces: Interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces, 15*(2), 161-176.

Dijkink, Speranza, Paltsidis, & Vereijken. (2007). Air dispersion of starch–protein mixtures: A predictive tool for air classification performance. *Powder Technology,172*(2), 113-119.

Du, Jiang, Yu, & Jane. (2014). Physicochemical and functional properties of whole legume flour. *LWT - Food Science and Technology, 55*(1), 308-313.

Duranti, & Gius. (1997). Legume seeds: Protein content and nutritional value. *Field Crops Research, 53*(1), 31-45.

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia, 77*, 67-82.

Ekvall, Stegmark, & Nyman. (2007). Optimization of extraction methods for determination of the raffinose family oligosaccharides in leguminous vine peas (*Pisum sativum L.*) and effects of blanching. *Journal of Food Composition and Analysis, 20*(1), 13-18.

Eldridge, A.C., Hall, P.K. and Wolf, W.J. 1963. Stable foams from unhydrolyzed soybean protein. *Food Technology.* 17: 1592.

Emami, Tabil, Tyler, & Crerar. (2007). Starch–protein separation from chickpea flour using a hydrocyclone. *Journal of Food Engineering, 82*(4), 460-465.

Endres, J. G. (2001). Definitions and methods of preparation. In *Soy protein products characteristics, nutritional aspects and utilization* (pp. 4-9). Urbana, Illinois: AOCS Press.

Ersland, D. R., Brown, J. W. S., Casey, R., and Hall, T. C., 1983, The storage proteins of *Phaseolus vutgaris L., Vicia Faba L.,* and *Pisum sativum L.*, in: "The Genetics and Biochemistry of Seed Proteins", W. Gottschalk and H. P. Müller, eds., Martinus Nijhoff, The Hague. In press.

Fao.org. (2015). Retrieved 22 November 2015, from http://www.fao.org/waicent/faoinfo/economic/faodef/fdef04e.htm#4.01

Fernández-Quintela, A., Macarulla, M., Del Barrio, T., & Martínez, S. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition,51*(4), 331-341

Foegeding, & Davis. (2011). Food protein functionality: A comprehensive approach. *Food Hydrocolloids, 25*(8), 1853-1864.

Frías, J., Martínez-Villaluenga, C., Gulewicz, P., Perez-Romero, A., Pilarski, R., Gulewicz, K., & Vidal-Valverde, C. (2007). Biogenic amines and HL60 citotoxicity of alfalfa and fenugreek sprouts. *Food Chemistry, 105*(3), 959-967.

Frias, Vidal-Valverde, Bakhsh, Arthur, & Hedley. (1994). An Assessment of variation for nutritional and non-nutritional carbohydrates in lentil seeds (*Lens culinaris*). *Plant Breeding, 113*(2), 170-173.

Gatehouse, J., Lycett, G., Croy, R., & Boulter, D. (1982). The post-translational proteolysis of the subunits of vicilin from pea (*Pisum sativum L.*). *The Biochemical Journal, 207*(3), 629-32.

Gatehouse, J., Croy, R., & Boulter, D. (1980). Isoelectric-focusing properties and carbohydrate content of pea (*Pisum sativum*) legumin. *The Biochemical Journal, 185*(2), 497-503

Guegen, J., & Barbot, J. (1988). Quantitative and qualitative variability of pea (*Pisum sativum L.*) protein composition. *Journal of the Science of Food and Agriculture, 42*, 209-224.

Hall, G. M. (1996). *Methods of testing protein functionality*. London, UK: Blackie Academic & Professional.

Han, J., & Khan.K. (1990b). Functional properties of pin-milled and air-classified dry edible bean fractions. *Cereal Chemistry, 67*, 390-394.

Harvey, D. G. (1970). In *Tables of the amino acids in foods and feeding stuffs*. Farnum Royal: Commonwealth Agricultural Bureaux

Hedley, C. L. (2001). Introduction: grain legume carbohydrates. In *Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics* (pp. 11). Oxon: CAB International.

Heywood, A., Myers, D., Bailey, T., & Johnson, L. (2002). Functional properties of extruded-expelled soybean flours from value-enhanced soybeans. *Journal of the American Oil Chemists' Society, 79*(7), 699-702.

Hilgenfeldt, S., Koehler, S., & Stone, H. (2001). Dynamics of coarsening foams: Accelerated and self-limiting drainage. *Physical Review Letters, 86*(20), 4704-7.

Hoover, Hughes, Chung, & Liu. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International,43*(2), 399-413.

Horan, F. (1974). Soy protein products and their production. *Journal of the American Oil Chemists' Society, 51*(1Part1), 67A-73A.

Horváth, E., Ormai-Cserhalmi, Z., & Czukor, B. (1989). Functional properties of air-classified yellow pea (*Pisum sativum*) fractions. *Journal of the Science of Food and Agriculture, 49*(4), 493-497.

Hove, E., King, S., & Hill, G. (1978). Composition, protein quality, and toxins of seeds of the grain legumes Glycine max, Lupinus spp., Phaseolus spp. Pisum sativum, and Vicia faba. *New Zealand Journal of Agricultural Research, 21*(3), 457-462.

Hua, Huang, Qiu, & Liu. (2005). Properties of soy protein isolate prepared from aqueous alcohol washed soy flakes. *Food Research International, 38*(3), 273-279.

Hutton, C., & Campbell, A. (1977). Functional properties of a soy concentrate and a soy isolate in simple systems and in a food system. *Journal of Food Science, 42*(2), 457-460.

ILDIS.ORG,. (2015). *Information about the Family Leguminosae*. Retrieved 12 September 2015, from http://www.ildis.org/Leguminosae/

Jansman AJM. (1996). Bioavailability of proteins in legume seeds. Grain Legumes (AEP) 11, p. 19.

Johansen, H. N., Glitsø, V. E., & Bach Knudsen, K. (1996). Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 44*(6), 1470-1474.

Johnson, D. (1976). Oilseed vegetable protein concentrates. *Journal of the American Oil Chemists' Society, 53*(6Part1), 321-324.

Kaldy, M. S., & Kasting, R. (1974). Amino acid composition and protein quality of eight fababean cultivars. *Canadian Journal of Plant Science, 54*, 869-871.

Kandlakunta, Rajendran, & Thingnganing. (2008). Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. *Food Chemistry, 106*(1), 85-89.

Karaca, Low, & Nickerson. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International, 44*(9), 2742-2750.

Kaur, M., & Singh, N. (2007). A comparison between the properties of seed, starch, flour and protein separated from chemically hardened and normal kidney beans. *Journal of the Science of Food and Agriculture, 87*(4), 729-737.

Karaca, Low, & Nickerson. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International, 44*(9), 2742-2750.

Kinsella, J. E. (1976). Functional properties of food proteins: a review. *Critical Reviews in Food Science and Nutrition*. 1976, 7, 219-280.

Kinsella, J. (1981). Functional properties of proteins: Possible relationships between structure and function in foams. *Food Chemistry, 7*(4), 273-288.

Kiosseoglou, V., & Paraskevopoulou, A. (2011). Functional and physicochemical properties of pulse proteins. In *Pulse Foods* (pp. 57-90). Elsevier.

King, J., Aguirre, C., & De PABLO, S. (1985). Functional properties of lupin protein isolates (*Lupinus albus cv Multolupa*). *Journal of Food Science,50*(1), 82-87.

Knudsen, K., & Li, B. (1991). Determination of oligosaccharides in protein-rich feedstuffs by gas-liquid-chromatography and high-performance liquid-chromatography. *Journal of Agricultural and Food Chemistry, 39*(4), 689-694

Koehler, S., Hilgenfeldt, S., & Stone, H. (2000). A generalized view of foam drainage: Experiment and theory. *Langmuir, 16*(15), 6327-6341.

Kosson, R. (1992). Determination of raffinose type sugars in seeds of leguminous plants by high pressure liquid chromatography (HPLC). *Roczniki Panstwowego Zakladu Higieny, 43*(2), 179-85.

Kudre, T., Benjakul, S., & Kishimura, H. (2013). Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *Journal of the Science of Food and Agriculture, 93*(10), 2429-2436.

Kuntz, I. (1971). Hydration of macromolecules. III. Hydration of polypeptides. *Journal of the American Chemical Society, 93*(2), 514-516.

Kroll, J. (1992), E. Dickinson: An Introduction to Food Colloids. 207. Oxford University Press, Oxford, New York, Tokyo 1992.

Laemmli, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature, 227*(5259), 680-5.

Lam, Y., & Lumen, B. (2003). Dietary importance. In *Encyclopedia of Food Sciences and Nutrition* (pp. 3529-3534). Oxford: Elsevier Science Ltd.

Lee, J., & Lopez, A. (1984). Modification of plant proteins by immobilized proteases. *Critical Reviews in Food Science and Nutrition, 21*(4), 289-322.

Liu, L., Hung, T., & Bennett, L. (2008). Extraction and characterization of chickpea *(Cicer arietinum)* albumin and globulin. *Journal of Food Science, 73*(5), C299-C305.

Liu, J., Yang, S., Lee, C., & DeVoe, D. (2008). Polyacrylamide gel plugs enabling 2-D microfluidic protein separations via isoelectric focusing and multiplexed sodium dodecyl sulfate gel electrophoresis. *Electrophoresis,29*(11), 2241-2250.

Lusas, E. W., & Rhee, K. C. (1995). Soy protein processing and utilisation. In *Practical Handbook of Soybean Processing and Utilization* (pp.117-146). St. Louis, Missouri: AOCS Press.

Lusas, E. W., & Hernandez, Ernest. (1997). Ethanol. In P. J. Wan, & P. J. Wakelyn (Eds.), *Technology and solvents for extracting oilseeds and nonpetroleum oils* (pp. 192-197). Illinois: AOCS Press.

Maquet, A., Vekemans, X., & Baudoin, J. (1999). Phylogenetic study on wild allies of Lima bean, *Phaseolus lunatus* (Fabaceae), and implications on its origin. *Plant Systematics and Evolution, 218*(1), 43-54.

Martin, Grolle, Bos, Stuart, & Van Vliet. (2002). Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability. *Journal of Colloid and Interface Science, 254*(1), 175-183

Matta, N. K., Gatehouse, J. A., & Boulter, D. (1981). Molecular and subunit heterogeneity of legumin of *Pisum sativum L*. (garden pea)- a multi-dimensional gel electrophoretic study. *Journal of Experimental Botany, 32*(6), 1295-1307.

Mathers, J. (2002). Pulses and carcinogenesis: Potential for the prevention of colon, breast and other cancers. *British Journal of Nutrition, 88*(S3), 273-279.

McCrory, M., Hamaker, B., Lovejoy, J., & Eichelsdoerfer, P. (2010). Pulse consumption, satiety, and weight management. *Advances in Nutrition, 1*(1), 17-30.

McWatters, K., & Holmes, M. (1979). Salt concentration, pH, and flour concentration effects on nitrogen solubility and emulsifying properties of peanut flour. *Journal of Food Science, 44*(3), 765-769.

Meng, G., & Ma, C. (2002). Characterization of globulin from *Phaseolus angularis* (red bean). *International Journal of Food Science & Technology, 37*(6), 687-695.

Meyer, E. W. (1825). Oilseed protein concentrates and isolates. *Journal of The American Oil Chemists' Society, 48*, 484-488.

Miyazawa, T., ITO, S., & Fungino, Y. (1975). Fatty acid composition of glycerides and stereo specific analysis of tryglyceride in pea seeds. *Journal of Nutritional Science and Vitaminology, 21*, 137-142.

Mosse, J., & Pernollet, J. C. (1983). Storage proteins of legume seeds. In *Chemistry and biochemistry of legumes* (pp. 111-193). London: Edward Arnold.

Moure, Sineiro, Domínguez, & Parajó. (2006). Functionality of oilseed protein products: A review. *Food Research International, 39*(9), 945-963.

Mudryj, A., Yu, N., & Aukema, H. (2014). Nutritional and health benefits of pulses. *Applied Physiology Nutrition and Metabolism, 39*(11), 1197-1204.

Mundi, & Aluko. (2012). Physicochemical and functional properties of kidney bean albumin and globulin protein fractions. *Food Research International, 48*(1), 299-306.

Murray, B. (2007). Stabilization of bubbles and foams. *Current Opinion in Colloid & Interface Science,12*(4), 232-241.

Naczk, M., Diosady, L., & Rubin, L. (1985). Functional properties of canola meals produced by a two-phase solvent extraction system. *Journal of Food Science, 50*(6), 1685-1688.

Nicorescu, Vial, Talansier, Lechevalier, Loisel, Della Valle, Riaublanc, Djelveh & Legrand. (2011). Comparative effect of thermal treatment on the physicochemical properties of whey and egg white protein foams. *Food Hydrocolloids, 25*(4), 797-808.

Nikmaram, N., Dar, B., Roohinejad, S., Koubaa, M., Barba, F., Greiner, R., & Johnson, S. (2017). Recent advances in γ-aminobutyric acid (GABA) properties in pulses: An overview. *Journal of the Science of Food and Agriculture, 97*(9), 2681-2689.

Norton. F. F.A. Bliss, and R. Bressani. 1985. Biochemical and nutritional attributes of grain legumes, p. 73-114. In: R.J. Summerfield and E.H. Roberts (eds.). Grain legume crops. Collins, London.

O'Kane, F., Vereijken, J., Gruppen, H., & Boekel, M. (2005). Gelation behavior of protein isolates extracted from 5 cultivars of *Pisum sativum L*. *Journal of Food Science, 70*(2), C132-C137.

O'Kane, F., Happe, R., Vereijken, J., Gruppen, H., & Van Boekel, M. (2004). Characterization of pea vicilin. 1. Denoting convicilin as the alpha-subunit of the Pisum vicilin family. *Journal of Agricultural and Food Chemistry, 52*(10), 3141-8.

Ohren, J. A. (1981). Process and product characteristics for soya concentrates and isolates. *Journal of American Oil Chemists' Society*, 58, 333-335.

ORR, M. L., & WATT, B. R. (1968). Amino acid content of foods.Home Economics Res.Rep.No. 4.,USDA, Washington D.C.

Osborne, T. B., & Campbell, G. F. (1898). Proteids of the pea. *Journal of the American Chemical Society*,*20*(5), 348-362.

Owusu-Ansah, Y., & Mccurdy, S. (1991). Pea proteins: A review of chemistry, technology of production, and utilization. *Food Reviews International, 7*(1), 103-134.

Paraman, I., Hettiarachchy, N., Schaefer, C., & Beck, M. (2006). Physicochemical Properties of Rice Endosperm Proteins Extracted by Chemical and Enzymatic Methods. *Cereal Chemistry*, *83*(6), 663-667.

Pattee, H., Salunkhe, D., Sathe, S., & Reddy, N. (1982). Legume lipids. *Critical Reviews in Food Science and Nutrition, 17*(2), 97-139.

Paulsson, & Dejmek. (1992). Surface film pressure of β-lactoglobulin, α-lactalbumin and bovine serum albumin at the air/water interface studied by wilhelmy plate and drop volume. *Journal of Colloid and Interface Science, 150*(2), 394-403.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry, 26*(3), 716-723.

Pelgrom, P. J. M., Vissers, A. M., Boom, R. M., & Schutyser, M. A. I. (2013). Dry fractionation for production of functional pea protein concentrates. *Food Research International, 53*(1), 232–239.

Pelgrom, P. J. M., Boom, R. M., & Schutyser, M. A. I. (2015a). Functional analysis of mildly refined fractions from yellow pea. *Food Hydrocolloids, 44*, 12–22

Perez-Hidalgo, M. A., Guerra-Hernández, E., & García-Villanova, B. (1997). Dietary fiber in three raw legumes and processing effect on chick peas by an enzymatic-gravimetric method. *Journal of Food Composition and Analysis, 10*(1), 66-72.

Phillippy, B. Q. (2003). Inositol phosphates in food. Advances in Food and Nutrition Research, 45, 1–60.

Pokatong, W. (1994). Effect of processing method on the composition and functionality of protein concentrates prepared from air classified pea protein. *M.Sc Thesis*. Saskatoon, SK: Department of Applied Microbiology and Food Science, University of Saskatchewan.

Pomeranz, Y. (1991) *Functional Properties of Food Components*. Second edition. San Diego: Academic Press Inc. (pp. 204-205) (Electronic) Available:

Quinn, J.R. and Paton, D. 1979. A practical measurement of water hydration capacity of protein materials. Cereal Chem. 56: 38-40.

Raatz, S.; The Bean Institute. [Online.] 2010. Nutritional Values of Dry Beans. Available from http://beaninstitute.com/health-benefits/nutritional-value-of-dry-beans/. [Accessed 1 June 2013.]

Rachwat, D., Nebesny, E., & Budryn, G. (2013). Chickpeas - composition, nutritional value, health benefits, application to bread and snacks: A review. *Critical Reviews in Food Science and Nutrition, 55*(8), 1137-1145.

Ragab, Babiker, & Eltinay. (2004). Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and/or salt concentration. *Food Chemistry, 84*(2), 207-212.

Ratnayake, Hoover, Shahidi, Perera, & Jane. (2001). Composition, molecular structure, and physicochemical properties of starches from four field pea (*Pisum sativum L.*) cultivars. *Food Chemistry, 74*(2), 189-202.

Rebello, C., Greenway, F., & Finley, J. (2014). Whole grains and pulses: A comparison of the nutritional and health benefits. *Journal of Agricultural and Food Chemistry, 62*(29), 7029-7049.

Reddy, Pierson, Sathe, & Salunkhe. (1984). Chemical, nutritional and physiological aspects of dry bean carbohydrates—A review. *Food Chemistry, 13*(1), 25-68.

Reichert, R. (1982). Air classification of peas (*Pisum sativum*) varying widely in protein content. *Journal of Food Science, 47*(4), 1263-1267.

Reinkensmeier, Bußler, Schlüter, Rohn, & Rawel. (2015). Characterization of individual proteins in pea protein isolates and air classified samples. *Food Research International, 76*(1), 160-167.

Rodríguez Patino, Carrera Sánchez, & Rodríguez Niño. (2008). Implications of interfacial characteristics of food foaming agents in foam formulations. *Advances in Colloid and Interface Science, 140*(2), 95-113.

Roy, F., Boye, J. I., & Simpson, B. K. (2010). Bioactive proteins and peptides in pulse crops: pea, chickpea and lentil. *Food Research International, 43*, 432-442.

Rui, X., Boye, J. I., Ribereau, S., Simpson, B. K., & Prasher, S. O. (2011). Comparative study of the composition and thermal properties of protein isolates prepared from nine Phaseolus vulgaris legume varieties. Food Research International, 44(8), 2497–2504.

Saarela, M. (2011). *Functional foods: Concept to product* (2nd ed., Woodhead Publishing in food science, technology, and nutrition; no. 205). Oxford; Philadelphia: Woodhead Pub.

Salunkhe, D. K., Kadam, S. S., & Chavan, J. K. (1985). Chemical composition. In *Post-harvest biotechnology of food legumes* (pp. 29-45). Florida: CRC Press.

Salunkhe, D. K., Kadam, S. S., & Chavan, J. K. (1985). Processing and utilization. In *Post harvest biotechnology of food legumes* (pp. 129). Florida: CRC Press

Sánchez-Mata, M. C., Peñuela-Teruel, M. J., Cámara-Hurtado, M. E., Díez-Marqués, C., & Torija-Isasa, M. (1998). Determination of mono-, di-, and oligosaccharides in legumes by high-performance liquid chromatography using an amino-bonded silica column. *Journal of Agricultural and Food Chemistry, 46*(9), 3648-3652.

Sathe, S., Deshpande, S., & Salunkhe, D. (1982). Functional properties of winged bean [*Psophocarpus tetragonolobus* (L.) DC] Proteins. *Journal of Food Science, 47*(2), 503-509.

Schutyser, & Van Der Goot. (2011). The potential of dry fractionation processes for sustainable plant protein production. *Trends in Food Science & Technology*, *22*(4), 154-164.

Schwenke, K. (1996). Food proteins. properties and characterization. Edited by S. Nakai and H. W. Modler. IX and 544 pages, numerous figures and tables. VCH Publishers, New York, Weinheim, Cambridge 1996. Price: 188.– DM. *Food / Nahrung,40*(6), 348.

Shapiro, M., and V. Galperin. "Air classification of solid particles: A Review." *Chemical Engineering & Processing: Process Intensification*, vol. 44, no. 2, 2005, pp. 279–285

Shand, Ya, Pietrasik, & Wanasundara. (2007). Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chemistry, 102*(4), 1119-1130.

Shukla, U. C., Dixit, M. L., & Arora, S. K. (1983). Mineral nutrition. In S. K. Arora (Ed.), *Chemistry and Biochemistry of Legumes* (pp. 259-276). New Delhi: Edward Arnold (Publishers) Limited.

Shukla, V. (1987). Quantitative Determination of Oligosaccharides in Defatted Soybean Products by High Speed Liquid Chromatography. *Lipid / Fett, 89*(2), 75-79.

Singh, Damanpreet, Singh, Bikram, & Goel, Rajesh Kumar. (2012). Role of saponins for the anticonvulsant effect of adventitious roots of Ficus religiosa. *Pharmaceutical Biology, 50*(7), 816-822.

Sikorski, Z.E., 2007. Chemical and Functional Properties of Food Components. 3rd Edn., CRC Press, Boca Raton, USA., ISBN-13: 978-0849396755, pp: 544.

Sikorski, Z.E., (Ed**.),** Functional properties of proteins in food systems. Chemical and functional properties of food proteins, Technomic Publishing Company, Inc, Lancaster, PA (2001), pp. 113-136

Singh, Balwinder, Singh, Jatinder Pal, Singh, Narpinder, & Kaur, Amritpal. (2017). Saponins in pulses and their health promoting activities: A review. *Food Chemistry, 233*, 540-549.

Smith, A., & Circle, S. (1938). Peptization of soybean proteins extraction of nitrogenous constituents from oil-bases with and without added slats. *Industrial & Engineering Chemistry,30*(12), 1414-1418.

Sosulski, F., Garrant, M. D., & Slinkard, A. E. (1976). Functional properties of ten legume flours. *Canadian Institute of Food Technology Journal, 9*, 66-69.

Sosulski, F., & McCurdy, A. (1987). Functionality of flours, protein fractions and isolates from field peas and faba bean. *Journal of Food Science,52*(4), 1010-1014.

Sosulski, F., & Youngs, C. (1979). Yield and functional properties of air-classified protein and starch fractions from eight legume flours. *Journal of the American Oil Chemists' Society, 56*(3Part2), 292-295.

Sreerama, Sashikala, Pratape, & Singh. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. *Food Chemistry, 131*(2), 462-468.

Stone, A., Karalash, A., Tyler, R., Warkentin, T., & Nickerson, M. (2015). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Research International, 76*, 31.

Stone, A., Avarmenko, N., Warkentin, T., & Nickerson, M. (2015). Functional properties of protein isolates from different pea cultivars. *24*(3), 827-833.

Subagio, A. (2006). Characterization of hyacinth bean (*Lablab purpureus* (L.) sweet) seeds from Indonesia and their protein isolate. *Food Chemistry, 95*(1), 65-70

Sumner, A., Nielsen, M., & Youngs, C. (1981). Production and evaluation of pea protein isolate. *Journal of Food Science, 46*(2), 364-366.

Swanson, B. (1990). Pea and lentil protein extraction and functionality. *Journal of American Oil Chemists' Society, 67*, 276-280.

Tahir, Vandenberg, & Chibbar. (2011). Influence of environment on seed soluble carbohydrates in selected lentil cultivars. *Journal of Food Composition and Analysis, 24*(4-5), 596-602.

Takayama, K., Muneta, P., & Wiese, A. (1965). Bean lipids-Lipid composition of dry beans and its correlation with cooking time. *Journal of Agricultural and Food Chemistry, 13*(3), 269-272.

Tiwari, B. K., & Singh, N. (2012). Protein isolates and concentrates. In *Pulse chemistry and technology* (pp. 85-95). Cambridge: The Royal Society of Chemistry.

Toews, R., & Wang, N. (2013). Physicochemical and functional properties of protein concentrates from pulses. *Food Research International,52*(2), 445-451.

Tyler, R T. (1982). Impact milling and air classification of grain legumes. *Ph. D Thesis*. Saskatoon, SK: Department of Food Science, University of Saskatchewan.

Tyler, R. T. (1984). Impact milling quality of grain legumes. *Journal of Food Science, 49*, 925-930.

Tyler, R.T., Youngs, C.G. and Sosulski, F.W. (1981) Air classification of legumes. 1. Separation efficiency, yield, and composition of the starch and protein fractions. Cereal Chemistry, 58, 144-148.

Tyler, R.T., Panchuk, B.D. Effect of seed moisture content on the air classification of field peas and faba beans. Cereal Chemistry, 59 (1982), pp. 31-33

Uzzan, A. (1988). Vegetable protein products from seeds: technology and uses in the food industry. In: Developments in Food Proteins (edited by B. J. F. Hudson). Pp. 73-1 18. New York Elsevier Applied science.

Vose, J. (1978). Separating Grain Components by Air Classification. *Separation and Purification Methods,7*(1), 1-29.

Wagner, J., & Guéguen, J. (1999). Surface functional properties of native, acid-treated, and reduced soy glycinin. 2. Emulsifying properties. *Journal of Agricultural and Food Chemistry, 47*(6), 2181-7.

Wang, N., Daun, J., & Malcolmson, L. (2003). Relationship between physicochemical and cooking properties, and effects of cooking on antinutrients, of yellow field peas (*Pisum sativum*). *Journal of the Science of Food and Agriculture, 83*(12), 1228-1237.

Wierenga, Van Norél, & Basheva. (2009). Reconsidering the importance of interfacial properties in foam stability. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 344*(1), 72-78.

Wilde, P. (2000). Interfaces: Their role in foam and emulsion behaviour. *Current Opinion in Colloid & Interface Science, 5*(3), 176-181.

Wilde, Mackie, Husband, Gunning, & Morris. (2004). Proteins and emulsifiers at liquid interfaces. *Advances in Colloid and Interface Science, 108*, 63-71.

Winham, D., Hutchins, A., & Johnston, C. (2007). Pinto bean consumption reduces biomarkers for heart disease risk. *Journal of the American College of Nutrition, 26*(3), 243-249

Withana-Gamage, T., Wanasundara, J., Pietrasik, Z., & Shand, P. (2011). Physicochemical, thermal and functional characterisation of protein isolates from Kabuli and Desi chickpea (*Cicer arietinum* L.): A comparative study with soy (*Glycine max*) and pea (*Pisum sativum L.*). *Journal of the Science of Food and Agriculture, 91*(6), 1022-31

Wright, D., Bumstead, M., Coxon, D., Ellis, H., DuPont, M., & Chan, H. (1984). Air classification of pea flour–analytical studies. *Journal of the Science of Food and Agriculture, 35*(5), 531-542.

Wolf, W. J. (197Ob). Soybean proteins: their functional, chemical and physical properties. *Journal of Agricultural and Food Chemistry, 18,* 969-976.

Yang, & Foegeding. (2010). Effects of sucrose on egg white protein and whey protein isolate foams: Factors determining properties of wet and dry foams (cakes). *Food Hydrocolloids, 24*(2), 227-238.

Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T., & Ishii, K. (1972). Whipping and emulsifying properties of soybean products. *Agricultural and Biological Chemistry, 36*(5), 719-727.

Yeh, C. T., & Yen, G. C. (2003). Effects of phenolic acids on human phenolsulfotransferases in relation to their antioxidant activity. *Journal of Agricultural and Food Chemistry*, 51, 1474–1479.

Yin, S., Tang, C., Wen, Q., & Yang, X. (2010). Functional and conformational properties of phaseolin (*Phaseolus vulgris* L.) and kidney bean protein isolate: A comparative study. *Journal of the Science of Food and Agriculture,90*(4), 599-607.

Youngs, C. G. (1975). Primary processing of pulse. In *Oilseed and pulse crops in Western Canada* (pp. 617-631). Saskatoon.

Zhang, Jiang, Mu, & Wang. (2009). Emulsifying properties of chickpea protein isolates: Influence of pH and NaCl. *Food Hydrocolloids,23*(1), 146-152.